CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

211801Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Cross Discipline Team Leader Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

NDA/BLA Multidisciplinary Review and Evaluation

Application Type	New Drug Application (NDA)
Application Number(s)	211801 (IND #108,732)
Priority or Standard	Standard
Submit Date(s)	09/12/2018
Received Date(s)	09/12/2018
PDUFA Goal Date	09/12/2019
Division/Office	Division of Gastroenterology and Inborn Errors Products (DGIEP)/ Office of Drug Evaluation III (ODE III)
Review Completion Date	09/10/2019
Established/Proper Name	Tenapanor (RDX5791; AZD1722)
(Proposed) Trade Name	Ibsrela
Pharmacologic Class	Sodium/hydrogen exchanger 3 (NHE3) inhibitor
Code name	
Applicant	Ardelyx, Inc.
Dosage form	Oral tablets
Applicant proposed Dosing Regimen	50 mg orally twice daily
Applicant Proposed Indication(s)/Population(s)	Treatment of Irritable Bowel Syndrome with Constipation (IBS-C) in Adults
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	440630006
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	Treatment of Irritable Bowel Syndrome with Constipation (IBS-C) in Adults
Recommended Dosing Regimen	50 mg orally twice daily

Table of Contents

Table of Tables

Table of Figures

Reviewers of Multidisciplinary Review and Evaluation

Regulatory Project Manager	Mary Chung
Nonclinical Reviewer	Dinesh Gautam
Nonclinical Team Leader	Sushanta Chakder
Office of Clinical Pharmacology Reviewer(s)	Sojeong Yi, Hyewon Kim
Office of Clinical Pharmacology Team Leader(s)	Jie (Jack) Wang
Clinical Reviewer	Elizabeth Mannick
Clinical Team Leader	Tara Altepeter
Statistical Reviewer	Shahla Farr
Statistical Team Leader	George Kordzakhia
Cross-Disciplinary Team Leader	Tara Altepeter
Division Associate Director (DGIEP)	Jessica J. Lee
Division Director (OCP)	Shirley Seo
Division Deputy Director (OB/DBIII)	Gregory Levin
Office Director (or designated signatory authority)	Victor Crentsil

Additional Reviewers of Application

OPQ	
OPQ/ATL:	Hitesh Shroff
OPQ/Drug Product	Hamid Shafiei/ Moo Jhong Rhee
OPQ/Drug Substance	Sukhamaya (Sam) Bain/ Donna Christner
OPQ/Process & Microbiology	Yuesheng Ye/ Nallaperumal Chidambaram
OPQ/Facility	Yuesheng Ye/ Vidya Pai
OPQ/Biopharmaceutics	Sarah Ibrahim/ Tapash Ghosh
OPQ/Environmental Analysis	Raanan Bloom
DPMH Pediatrics	Carolyn Yancey/ Hari Sachs
DPMH Maternal	Christos Mastroyannis/ Tamara Johnson
OSI/DCCE	Susan Leibenhaut/ Susan Thompson
QT IRT	Nan Zheng, Yuan Xu, Janelle Chen, Dalong Huang, Mohammad Rahman, Michael Li, Jose Vicente Ruiz, Lars Johannesen/ Christine Garnett
COA	Susan Pretko/ Sarrit Kovacs
DCaRP Cardiology	Stephen (Steve) Grant/ Marty (Martin) Rose
DCaRP Renal	Shen Xiao/ Aliza Thompson
OSE/DEPI	Monisha Billings/ Patricia Bright
OSE/DRISK	Yasmeen Abou-Sayed/ Donella Fitzgerald

OSE/DPV	Michelle Hines/ Lisa Harinstein
DMPP/PLT	Maria Nguyen/ Marcia Williams/ LaShawn Griffiths
OPDP	Meeta Patel

OPQ = Office of Pharmaceutical Quality
ATL= Application Team Lead
DPMH= Division of Pediatric and Maternal Health
QT IRT= QT Interdisciplinary Review Team
OPDP = Office of Prescription Drug Promotion
OSI = Office of Scientific Investigations
DCCE= Division of Clinical Compliance Evaluation
OSE = Office of Surveillance and Epidemiology
DEPI = Division of Epidemiology
DMEPA = Division of Medication Error Prevention and Analysis
DRISK = Division of Risk Management
DMPP= Division of Medical Policy Programs
PLT= Patient Labeling Team
DPV= Division of Pharmacovigilance
DaCRP= Division of Cardiovascular and Renal Products
COA= Clinical Outcome Assessments Staff

Glossary

ACE	antegrade continent enema
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
BID	twice daily
BILI	bilirubin
BLA	biologics license application
BMI	body mass index
CFR	Code of Federal Regulations
CI	confidence interval
CIC	chronic idiopathic constipation
CKD	chronic kidney disease
C_{max}	maximum concentration
CMC	chemistry, manufacturing, and controls
CMH	Cochran-Mantel-Haenszel
CRD	colorectal distension
CSBM	complete spontaneous bowel movement
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P540
DDI	drug-drug interaction
DGIEP	Division of Gastroenterology and Inborn Errors Products
DILI	drug-induced liver injury
DHOT	Division of Hematology Oncology Toxicology
ECG	electrocardiogram
ESRD	end-stage renal disease
FDA	Food and Drug Administration
GI	gastrointestinal
GD	gestation day
GLP	good laboratory practice
HEK	human embryonic kidney
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome with constipation
IND	investigational new drug
ISE	integrated summary of efficacy
ISS	integrated summary of safety
ITT	intent to treat
IVRS	interactive voice response system

MedDRA	Medical Dictionary for Regulatory Activities
NDA	new drug application
NOAEL	no adverse effect level
OK	opossum kidney
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PD	pharmacodynamics
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PND	postnatal day
PP	per protocol
PREA	Pediatric Research Equity Act
PT	preferred term
QD	once daily
RW	randomized-withdrawal
SAE	serious adverse event
SOC	system organ class
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
VMR	visceral motor reflex

1. Executive Summary

1.1. Product Introduction

IBSRELA (tenapanor hydrochloride), a new molecular entity, is a first-in-class, orally administered, small molecule, inhibitor of the sodium-hydrogen exchanger 3 (NHE3). The proposed dosage is 50 mg BID for the treatment of adults with constipation predominant irritable bowel syndrome (IBS-C).

NHE3 is also known as SLC9A3 and Na+/H+ exchanger 3. NHE3 is expressed on the luminal side of stomach and intestinal epithelial cells as well as in the kidney, gallbladder, ovary, thymus and prostate. It is one of nine sodium/hydrogen antiporters of the NHE family (Donowitz et al. 2013). It regulates sodium and water absorption and pH by exchanging extracellular sodium for intracellular hydrogen ions. Inhibition of NHE3 results in increased secretion of water into the intestinal lumen and looser bowel movements. NHE3 is closely coupled to other apical transporters, including: down regulated in adenoma (DRA, also known as SLC26A3), the sodium-coupled glucose transporter (SGLT1) and the cystic fibrosis transmembrane regulator (CFTR) (Musch et al. 2009; Jakab et al. 2011; Girardi and Di Sole 2012). Guanylate cyclase and bacterial toxins that upregulate guanylate cyclase (as seen in cholera, enteropathogenic Escherichia coli and C. difficile infections) inhibit NHE3, contributing to their pro-secretory activity (Hayashi et al. 2004; Hecht et al. 2004; Subramanya et al. 2007; McHugh et al. 2018). Tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) also inhibit NHE3 (Amin et al. 2006).

Mice with targeted deletion of the NHE3 gene have a shortened lifespan due to a syndrome of chronic diarrhea, abdominal distention, metabolic acidosis and hyponatremia (Woo et al. 2002). Knock-out mice also develop distal colitis characterized by neutrophil infiltration and depletion of goblet cells. Alkalization of the gut may alter the microbiome in these animals (Larmonier et al. 2013). In humans, patients with inactivating mutations in the NHE3 gene develop congenital sodium diarrhea in infancy, and if they survive, are predisposed to inflammatory bowel disease when older. The same phenotype is seen in patients with activating mutations in guanylate cyclase (Janecke et al. 2016).

1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant provided evidence from two confirmatory trials (Study 301 and 302) that provide substantial evidence of effectiveness and support approval of tenapanor 50 mg BID for the treatment of IBS-C.

Both studies were multicenter, randomized, double-blind, placebo controlled trials designed to assess the efficacy and safety of tenapanor in the treatment of IBS-C. Study 301 included a 12-week, double-blind, parallel group treatment period, followed by a 4-week randomized withdrawal period. Study 302 included a 26-week, double-blind, parallel group period, and provided data which demonstrated that efficacy is maintained with chronic treatment past 12 weeks. The primary endpoint in both trials was the proportion of patients who were overall

responders over the first 12 weeks. An overall responder was defined as achieving success on both the "abdominal pain weekly responder" and "CSBM [complete spontaneous bowel movement] responder" components of the endpoint for at least 6 of the first 12 weeks. This endpoint was consistent with FDA's published guidance on IBS drug development (May 2012).

The abdominal pain response criterion was defined as a decrease of 30% or more of percent change in average weekly worst abdominal pain score from baseline. The average weekly abdominal pain score was calculated as the average score for all days during a valid week, using a 0 to 10 numeric rating scale.

The CSBM response criterion was defined as an increase of one or more in average weekly CSBMs from baseline. The average weekly CSBMs was calculated as the sum of the number of CSBMs reported during each day of the defined weekly period divided by the number of days CSBMs were reported multiplied by seven.

In Study 301, 606 patients were randomized 1:1 to received tenapanor or placebo. In the tenapanor group, 27% of patients were overall responders, compared with 19% on placebo. Similarly, in Study 302, 593 patients were randomized, and 37% of tenapanor treated patients were overall responders, compared with 24% in the placebo group. Secondary endpoints in Study 302 included, as a multiplicity controlled endpoint, the proportion of patients who were overall responders for 9/12 weeks (14% for tenapanor vs 3% for placebo). In addition, as an exploratory endpoint, the proportion of patients who were overall responders for 13/26 weeks was also reported (36% on tenapanor vs 24% on placebo), which helped to support that the initially demonstrated benefit was continued with longer term therapy. Details of additional secondary endpoints assessed are reviewed in Section 9.1.2.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Tenapanor is a new molecular entity, a first-in-class inhibitor of the sodium-hydrogen exchanger 3 (NHE3). Tenapanor represents a new treatment option with a novel mechanism of action, to complement the arsenal of approved therapies for patients with IBS-C.

IBS-C affects a large population and there is a significant unmet medical need for the patients. The prevalence of IBS-C in North America is 5%, affecting females more frequently than males (approximately 2 to 3:1 female predominance (Saito et al. 2002)). IBS-C is a multi-symptom disease with a significant and growing public health burden, due to significant loss in productivity and serious, disabling symptoms experienced by a small subset of the more severely affected patients. There are currently four drugs approved in the United States for the treatment of IBS-C. Lubiprostone (Amitiza), approved for IBS-C on April 29, 2008, activates type-2 chloride channels in gastrointestinal (GI) tract epithelial cells to increase secretion of fluid in the intestine, and the indication is limited to use in women. Two of the other approved treatments for IBS-C are guanylate cyclase-C agonists, including linaclotide (Linzess), approved on August 30, 2012, and plecanatide (Trulance), approved on January 24, 2018. Additionally, tegaserod (Zelnorm), a serotonin receptor 4 (5-HT4) agonist, which was previously withdrawn from the market secondary to cardiovascular risks, was recently re-introduced to the market with a revised indication restricted to women less than 65 years of age, on March 29, 2019. All the available alternative therapies demonstrate a modest treatment effect, so there remains a need for additional therapeutic options for patients with IBS-C.

Tenapanor was demonstrated to be effective for the treatment of adults with IBS-C in two adequate and well controlled trials (Study 301 and Study 302) that were submitted with this application. A single dose (50 mg BID) of tenapanor was evaluated in both trials. The primary endpoint in both trials was the proportion of patients who were “overall responders” for at least 6 of the first 12 weeks. For the overall responder endpoint, a subject was required to achieve at least a 30% reduction from baseline in average of the worst daily abdominal pain score, and an increase of at least 1 complete spontaneous bowel movement from baseline, both in the same week, for at least 6 of the 12 weeks. A greater proportion of tenapanor treated patients achieved this endpoint compared to placebo in both trials. In study 302, all key secondary endpoints achieved statistical significance. However, a benefit was not shown on the first ranked secondary endpoint (Overall CSBM response 6/12 weeks) in Study 301; thus, sequential testing of other endpoints was stopped.

The safety profile of tenapanor supports approval. Adverse events (AEs) occurred primarily in the gastrointestinal tract. Diarrhea was the most common AE, occurring in approximately 15% of treated patients (compared to 2% on placebo) over the 12-week treatment period in Study 301. Similarly, diarrhea was reported in 16% of tenapanor treated patients (compared to 4% on placebo) over the 26-week treatment period in Study 302. Across the two phase 3 studies, severe diarrhea occurred in 2.5% of all tenapanor treated patients (compared to 0.2% on placebo) and diarrhea was the most common AE leading to discontinuation. Serious AEs were rare and mostly unrelated to study drug, with

exception of a single SAE related to severe diarrhea. Labeling will reflect the risk of diarrhea. There is a theoretical concern that tenapanor may induce colitis or inflammatory bowel disease. This is hypothesized to be a risk based on two sets of findings. First, knockout mice deficient in NHE3 develop inflammatory bowel disease (IBD), and mice studied in murine models of IBD have reduced expression of NHE3 (Barmeyer et al. 2004; Kiela et al. 2009; Larmonier et al. 2011; Lenzen et al. 2012; Larmonier et al. 2013; Kumar et al. 2016; Harrison et al. 2018). Second, human patients with inactivating mutations of NHE3 or activating mutations in guanylate cyclase (closely related) develop congenital sodium diarrhea (in infancy) and those who survive have an increased risk of inflammatory bowel disease (Engevik et al. 2015; Janecke et al. 2015). Within the tenapanor safety database submitted with this new drug application (NDA), there is no clear signal of new onset colitis or inflammatory bowel disease. There were eight cases of rectal bleeding reported (seven on treatment, one on placebo), but none were clearly determined to be due to colitis. Diarrhea was commonly observed in tenapanor treated patients, which was expected given the pharmacological effect of the drug. However, given that diarrhea can also be a presenting sign of colitis, and that the majority of patients did not undergo colonoscopy with biopsy to confirm or refute a possible colitis diagnosis, uncertainty exists. It was reassuring, however, that most patients with diarrhea either were not so severely affected as to discontinue drug, or had resolution of the diarrhea after discontinuation of therapy. A signal detection study will be performed in Sentinel, post-approval, for continued monitoring of the potential for tenapanor to induce inflammatory bowel disease. Hyperkalemia was also noted as a laboratory abnormality occurring more frequently in tenapanor treated patients than placebo in one of the phase 3 trials. Laboratory shifts in hyperkalemia within the IBS-C trial were mostly regarded as not clinically significant by investigators. However, in a trial conducted under a separate IND in patients with chronic kidney disease, a differential risk of reported AEs of hyperkalemia was noted, as well as 2 SAEs in tenapanor treated patients who developed hyperkalemia requiring hospitalization. This information will be noted in labeling.

Uncertainties exist regarding the safety and potential risk-benefit profile in both pediatric patients and in breast feeding infants whose mothers are treated with tenapanor. There are no clinical data to date in pediatric patients treated with tenapanor. In the nonclinical program, findings in young juvenile rats (less than 1 week old animals, human age equivalent <2 years) demonstrated premature mortality at doses as low as 0.3 mg/kg/day, some occurring as early as Postnatal Day 8. There are no additional juvenile animal data available that directly address human age equivalence of $\geq$2 years to <12 years. An additional juvenile animal toxicity study in rats, with dosing initiated at Postnatal Day 21 (to cover pediatric patients between the ages of 2 to <12 years of age) will be required as a postmarketing study under the Pediatric Research Equity Act (PREA). This study will help to inform if a dose can be identified with an adequate safety margin to support the study of tenapanor in pediatric patients ages 6 years and older (given that 6 years is the lowest age that IBS can be reliably diagnosed in pediatrics). For this reason, tenapanor is currently contraindicated in pediatric patients less than 6 years of age, until more information is available. The label includes a boxed warning with a contraindication for use in pediatric patients up to 6 years of age, and a warning to avoid use in pediatric patients age 6 to <12 years of age. A milk-only lactation study will also be required to determine the safety of tenapanor for breast-fed infants whose mothers are receiving therapy.

In summary, the available data support a favorable benefit-risk assessment and approval of tenapanor for treatment of IBS-C in adults. Although the magnitude of treatment effect demonstrated was modest, the safety profile appears relatively benign, with

diarrhea as the most commonly occurring adverse event. Diarrhea is an adverse event that patients can easily self-identify, and therefore, this risk can be mitigated. Approval of tenapanor may assist in alleviating an unmet need for IBS-C patients.

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application

X	The patient experience data that was submitted as part of the application, include:		Section where discussed, if applicable
	X	Clinical outcome assessment (COA) data, such as	
		X Patient reported outcome (PRO)	-primary endpoint includes patient reported outcome of pain and stool frequencies directly reported by patients (discussed in section 9) -IBS-eDiary contained additional concepts which were reviewed but not included in labeling (discussed in section 4.3 below) -applicant also assessed IBS-QOL, eight subscales and overall scores (Section 11.4.1.2.2.29 of Clinical Study Report [CSR] for Study 301, Section 11.4.1.2.2.29 of CSR for Study 302) which are not further described in the review as they were neither multiplicity controlled not considered relevant to support review decision.
		□ 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)	
□	Patient experience data that was not submitted in the application, but was considered in this review.		

2. Therapeutic Context

2.1. Analysis of Condition

Irritable bowel syndrome (IBS) is not one, narrowly defined, illness but instead, a constellation of symptoms, the frequency and severity of which can vary among individuals. It results in chronic abdominal pain and altered defecation in the absence of other etiologies. No one treatment is effective in all, or even most, patients with IBS. The etiology is multifactorial and includes disordered motility, psychosocial stress, visceral hypersensitivity, carbohydrate intolerance, bacterial overgrowth/dysbiosis, immunological abnormalities (mast cell activation, postinfectious IBS) and bile acid malabsorption (Enck et al. 2016). IBS has been historically defined by a specific set of diagnostic criteria (the Rome Criteria), currently in its fourth iteration (Table 1). Four subcategories of IBS exist: irritable bowel syndrome with constipation (IBS-C), irritable bowel syndrome with diarrhea (IBS-D), irritable bowel syndrome-mixed (IBS-M) with both diarrhea and constipation, and unsubtyped IBS (IBS-U) (Table 2). Once the genetics of IBS are more fully elucidated, it is likely that further subtypes will emerge.

Table 1. Rome IV Diagnostic Criteria for Irritable Bowel Syndrome

Recurrent abdominal pain, on average, at least 1 day per week in the last 3 months, associated with ≥ 2 of the following criteria:
1. Related to defecation.
2. Associated with a change in frequency of stool.
3. Associated with a change in form (appearance) of stool.

[a] Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis.

Table 2. Rome IV Irritable Bowel Syndrome-Subtypes Criteria

IBS Subtype	Criteria
IBS-C	More than one-fourth (25%) of bowel movements with Bristol Stool Scale Types 1-2 and less than one-fourth (25%) with Types 6-7.
IBS-D	More than one-fourth (25%) of bowel movements with Bristol Stool Scale Types 6-7 and less than one-fourth (25%) with Types 1-2.
IBS-M	More than one-fourth (25%) of bowel movements with Bristol Stool Scale Types 1-2 and more than one-fourth (25%) with Types 6-7.
IBS-U	Patients meet diagnostic criteria for IBS, but their bowel habits cannot be accurately categorized in any of the above subtypes.

IBS, irritable bowel syndrome; IBS-C, IBS with predominant constipation; IBS-D, IBS with predominant diarrhea; IBS-M, IBS with mixed bowel habits; IBS-U, unclassified IBS.
Source: Reviewer's table adapted from (Mearin et al. 2016)

Irritable bowel syndrome is a prevalent condition. IBS affects between 10 to 15% of the population in the United States and, approximately, one-third of these patients have IBS-C. More women than men suffer from IBS (odds ratio of 1.67) and the condition becomes less prevalent in adults over age 50 (Lovell and Ford 2012). No proven difference in prevalence among different racial and ethnic groups exists, although this issue has not been well studied. One study demonstrated a higher prevalence of IBS in Caucasians compared to African Americans (Wigington et al. 2005). Epidemiologic studies in Asia show increasing prevalence of IBS, correlated to socioeconomic status (Gwee et al. 2009).

Although IBS rarely results in death or permanent disability, the associated morbidity, emotional distress and cost to individuals and society is high. For gastrointestinal disease in the United States, estimated total burden of illness from IBS is second only to that from gastroesophageal reflux disease, amounting to at least $1.7 billion in direct costs and $20 billion in indirect costs (Hulisz 2004). In one survey, patients with IBS-C missed 0.8 days of work per month and had, on average, 5 days of disrupted productivity per month because of their symptoms (Heidelbaugh et al. 2015).

For an individual patient with IBS-C, quality of life can be impaired by intrusive sensations of abdominal pain, bloating, gas, incomplete evacuation and abdominal spasming that feed into anxiety and depression in a vicious cycle. In 2019, a small but significant minority of patients are still being referred for antegrade continent enema (ACE) procedures and total colectomy for intractable IBS-C, and many more undergo, possibly unnecessary, gynecological surgeries in attempts to relieve their pelvic pain (Longstreth 2007). The need to develop new therapies in this area is pressing, as many patients continue to experience refractory symptoms despite approved therapies.

2.2. Analysis of Current Treatment Options

Current treatment options for IBS-C, both FDA-approved and nonapproved, are listed in Table 3 and discussed below.

Table 3. Current Treatments for IBS-C

Product (s) Name	Relevant Indication	Year of Approval (for IBS-C)	Dosing/ Administration	Adverse Effects
FDA-approved: Lubiprostone	IBS-C (limited to women)	2008	8 mcg capsules by mouth BID	Common: Nausea Dyspnea
Linaclotide	IBS-C	2012	145 mcg capsules by mouth BID; 290 mcg capsules by mouth BID	Common: Diarrhea Rare: Gastrointestinal bleeding
Plecanatide	IBS-C	2018	3-mg tablet by mouth once daily	Common: Diarrhea
Tegaserod	IBS-C	2002-2007; re-introduced in limited population 2019.		Common: Diarrhea Rare: Cardiovascular and psychiatric
Dicyclomine	IBS	1950	10-20 mg capsules by mouth BID-QID	Common: Dizziness, dry mouth, blurred vision Rare: Cardiovascular and CNS

Product (s) Name	Relevant Indication	Year of Approval (for IBS-C)	Dosing/ Administration	Adverse Effects
Not FDA- approved: Amitriptyline	IBS	NA*	10-50-mg tablets by mouth once daily	Common: Constipation, dry mouth, drowsiness Rare: Potential for increased suicide risk, Glaucoma, Cardiac arrhythmias
Polyethylene glycol (PEG) 3350	IBS-C	NA	17 grams (1 capful) of powder in 4-8 oz liquid by mouth daily	Common: Diarrhea Rare: Possible neurological events
Senna	IBS-C	NA	8.6 g tablets, 2-4 by mouth once or twice daily	Common: Diarrhea Melanosis coli Dependency Rare: Possible cancer risk
Lactulose	IBS-C	NA	15-30 ml by mouth once to twice daily	Common: Bloating, gas, diarrhea
Magnesium	IBS-C	NA	500-1500 mg by mouth daily	Common: Diarrhea, cramping
Peppermint oil	IBS	NA	0.2-0.4 ml by mouth TID as oil or enteric-coated capsules	Common: GERD Anal Burning Rare: Worsening gallbladder disease
Psyllium husk	IBS	NA	5-10 grams by mouth one to three times daily	Common: Bloating and gas
Probiotics	IBS	NA	Multiple dosing regimens for multiple brands	Common: Bloating and gas Rare: Septicemia Headaches Histamine reactions
Low FODMAP diet	IBS	NA	NA	Common: Constipation (from eliminating fiber) Rare: Disordered eating
Hypnotherapy	IBS	NA	NA	None
Cognitive behavioral therapy	IBS	NA	NA	None
Yoga	IBS	NA	NA	**Rare:** injury

Abbreviations: BID, twice daily; FODMAP, fermentable oligo-, di-, mono-saccharides and polyols; GERD, gastroesophageal reflux disease; IBS-C, irritable bowel syndrome with constipation; NA, not applicable, QID, four times daily.
Source: Reviewer's table

FDA-Approved Treatments for IBS-C

Secretagogues:

Lubiprostone (Amitiza) is a chloride channel activator approved for the treatment of IBS-C in women 18 years of age and older in 2008 based on phase 3 trials enrolling more than 90% women. It activates the intestinal ClC-2 chloride channel, resulting in fluid secretion that lubricates the bowel and promotes motility. A dose-dependent increase in fetal loss occurred in pregnant guinea pigs at doses close to those used in clinical practice, which may limit its use in women of child-bearing age.

Linaclotide (Linzess) is a guanylate cyclase C receptor agonist. It was FDA approved for the treatment of IBS-C in adults in 2012. Activation of guanylate cyclase C results in an increase in intracellular cyclic guanosine monophosphate (cGMP), which activates the CFTR and inhibits NHE3, resulting in the secretion of chloride and water into the intestinal lumen. It has been associated with rectal bleeding and melena in less than 1% of patients in clinical trials. In addition, because of reports of deaths in neonatal and juvenile mice from diarrhea and dehydration at doses between 10 to 500 mcg/kg, the drug is not recommended in pediatric patients and is contraindicated in patients less than 6 years of age.

Plecanatide (Trulance) is a second drug in the class of guanylate cyclase C receptor agonists. It has a similar mechanism of action to linaclotide and was FDA approved for the treatment of IBS-C in January 2018. Plecanatide has a similar safety and efficacy profile to linaclotide. Based on animal studies, which showed mortality in juvenile mice after single doses of 0.5 mg/kg and higher, its use in pediatric patients is not recommended, and it carries a contraindication for patients less than 6 years of age.

Pro-Motility Agents:

Tegaserod (Zelnorm) is a 5-hydroxytryptamine 4 (5-HT_4) receptor partial agonist and 5-HT_{2B} antagonist that works on the serotonergic nervous system in the gastrointestinal tract to stimulate motility. Tegaserod was FDA-approved for short-term use in women with IBS-C in 2002. Novartis withdrew tegaserod from the market in 2007 after a large, postmarketing study determined that the number of cases of heart attack or stroke was higher in patients treated with tegaserod (13 of 11,614) than in controls (1 of 7031). On March 29, 2019, tegaserod was reintroduced to the U.S. market for use in adult women <65 years of age.

Dicyclomine hydrochloride (Bentyl) is an anti-cholinergic, anti-spasmodic agent that is FDA-approved for the treatment of irritable bowel syndrome. It acts as a smooth muscle relaxant in the large bowel, decreasing pain associated with colonic spasming. Because dicyclomine can cause constipation, it is often not a first-line treatment for IBS-C.

Non-FDA Approved Treatments for IBS-C

As indicated in Table 3 above, additional treatments that are not FDA-approved are commonly used in clinical practice. A complete discussion of these alternative therapies is beyond the scope of this review.

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Tenapanor (also known as AZD1722 and RDX 5791) is a new molecular entity and is not currently marketed in the U.S. The drug was initially developed for the treatment of IBS-C; investigational new drug (IND) application 108732 was opened by Ardelyx, Inc. on October 21, 2010. (b) (4)

(b) (4)

3.2. Summary of Presubmission/Submission Regulatory Activity

The Applicant met with the Division of Gastroenterology and Inborn Errors Products (DGIEP) during the IBS-C development program. Key recommendations and points of discussion follow.

Pre-IND Meeting (July 21, 2010):

- The Division stated that basing doses for phase 1 clinical studies on the no adverse effect level (NOAEL) from the 7-day rat toxicology study was unacceptable. The Applicant indicated plans to perform 28-day rat and dog studies, as well as in vitro metabolic stability and metabolite identification studies in liver microsomes, and protein binding studies in plasma.
- The Applicant asked for agreement from the Division that soft feces and mild diarrhea observed in 7-day rat and dog studies were drug-related pharmacological effects and not adverse events. The Division responded by saying that review of study reports would be necessary to make this determination. FDA noted that to classify the changes in goblet cells as an expected, drug-related pharmacological effect, the histopathology could not be irreversible.

- The FDA agreed to the Applicant's proposal to [redacted] (b) (4) The FDA commented that with the proposed allowance of [redacted] (b) (4) of related substances as impurities, the impurities would need to be monitored individually.

End-of-Phase 2 (EOP2) Meeting (June 9, 2015):

- FDA gave advice regarding further characterization of the M1 metabolite (16% of plasma drug-related substance). The Division requested a repeated dose toxicity study, embryo-fetal development study, and a carcinogenicity study in one species with the M1 metabolite. The Applicant responded that, in lieu of a repeated dose toxicity study, they planned to quantify M1 in plasma samples from phase 2 patients and calculate the ratio of exposure multiples to M1 in the dog to human M1 exposure at the anticipated dose of 50 mg BID. For reproductive toxicity, the Applicant planned to calculate exposure multiples to M1 in the rabbit, rat and mouse divided by human exposure. The Applicant stated that they would conduct further reproductive toxicity studies only if neither species provided a margin of 0.5. The Applicant agreed to perform carcinogenicity study in Tg.rasH2 mice to assess the carcinogenic potential of M1.
- The Division stated that the potential effects of hepatic impairment on pharmacokinetics (PK) and safety of tenapanor should be addressed because tenapanor is metabolized upon absorption. The Applicant agreed to provide additional data with a plan for further clinical pharmacology studies.
- The Division disagreed with [redacted] (b) (4)
- The Division agreed that a thorough QT study (tQT) was not necessary because nonclinical and clinical data showed no signal of QT prolongation.
- The Applicant and the Division agreed on the proposed dose (50 mg BID), phase 3 study designs, the primary endpoint (percent overall responder where a responder is defined based on simultaneous improvement in complete spontaneous bowel movement (CSBM) and abdominal pain for 50% of weeks) and secondary endpoints. The Agency recommended performing sensitivity analyses that evaluate greater reductions in abdominal pain intensity with treatment. The Applicant agreed to examine the cumulative distribution of several magnitudes of abdominal pain intensity reduction as a secondary endpoint.
- FDA did not agree with the plan [redacted] (b) (4)
- FDA stated that [redacted] (b) (4)
- FDA agreed with a deferral to conduct pediatric studies until establishment of safety and efficacy in adult IBS-C patients and a waiver for children less than 6 years of age, pending submission of the initial pediatric study plan (iPSP).

- The Applicant clarified that tenapanor exerts its effect on the NHE3 protein (not transcription) and that no evidence of tachyphylaxis exists.

Type C Guidance meeting, Chemistry, Manufacturing, and Controls/Clinical Pharmacology focused (October 31, 2016):

Refer to the detailed Office of Pharmaceutical Quality (OPQ) review for further details on manufacturing process issues and agreements. In summary, drug substance limits for impurities were agreed for (b) (4), and general unspecified impurity. A stability testing plan was agreed upon. The FDA agreed that the drug product impurity profile was adequately supported by good laboratory practice (GLP) toxicology studies and sufficiently characterized. The Applicant agreed that the validation report and data necessary to support the use of the high performance liquid chromatography impurity and assay method on the drug product would be provided in the NDA.

Type B Pre-NDA Meeting (May 1, 2018):

- FDA stated that the proposed integration strategy for the Integrated Summary of Efficacy (ISE) including data from two phase 3 trials and a supportive phase 2b trial appeared reasonable. FDA also agreed with separate presentation of efficacy data from the phase 2a trial since it differed in duration and dose from the other trials. FDA opined that the proposal to include a "Comparison of Results of Individual Studies" section for the ISE trials was reasonable. However, the FDA stated that its primary efficacy assessment would utilize the results of individual trials.
- FDA agreed to a "Comparison of Results in Subpopulations" section in the analysis of the pooled ISE data, provided the results not be used for labeling claims.
- FDA agreed with the proposed integration strategy for the Integrated Summary of Safety (ISS) across the 19 completed trials, to be analyzed as four separate analyses (Core Safety Analysis Set including phase 2 and 3 trials, ESRD Supportive Safety Analysis Set, CKD (chronic kidney disease) Supportive Safety Analysis Set, and Healthy Volunteers Supportive Safety Analysis Set. However, the FDA stated that its primary safety assessment would rely upon the safety data provided for the two phase 3 trials conducted in patients with IBS-C.
- FDA agreed that the exposure numbers presented with the ISS appeared acceptable for evaluation of the long-term safety of tenapanor. However, FDA noted that the long-term safety data were obtained from an open-label, long-term safety study (TEN-01-303) and that this issue would be re-evaluated during NDA review.
- FDA agreed to the proposed approach to analyze the prespecified secondary endpoint using a sequential testing procedure to control type 1 error.
- FDA did not agree to (b) (4) The FDA agreed to include only patients with moderate hepatic impairment in the study.

- FDA agreed that the completed drug-drug interaction (DDI) studies could support the NDA submission. It recommended conducting an additional DDI study with a concomitant, strong, CYP3A4/5 (cytochrome P450 3A4/5) inhibitor to address the potential effects on the PK of tenapanor and M1, as tenapanor and M1 are substrates of CYP3A4/5 in vitro.
- FDA stated that the qualification and control of impurities in the drug product and substance specifications and the proposed dissolution method appeared adequate.
- FDA recommended including a (b) (4) test in the drug substance specification at release and stability.

Type C Guidance Meeting (Written Responses Only June 19, 2018):

- FDA stated that the Applicant did not need to submit the cardiorenal-specific, primary pharmacology studies for the IBS-C application, but needed to submit secondary pharmacology studies regardless of indication.
- FDA stated that the nonclinical data appeared to support the safety of the levels of metabolite M1 observed in clinical trial batches.
- FDA stated that the nonclinical data appeared to support the safety of the tenapanor impurity profile.

Initial pediatric study plan (iPSP) was agreed on September 25, 2017. Refer to Section 11 below for details of the planned pediatric development program.

A Special Protocol Assessment request was submitted on January 6, 2015 for "A 26-week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CbyB6F/TgrasH3 Hemizygous Mice." It was reviewed by the Carcinogenicity Assessment Committee and accepted on November 16, 2016, following committee recommendations to increase the proposed doses.

An Amendment to the Special Protocol Assessment was submitted on March 10, 2017. The Applicant submitted an amendment to request lowering the high dose, male tenapanor groups and high dose, male and female M1 groups due to decreases in body weight gain over 71 days. The FDA agreed that the proposal for reduction of the high dose of AZD13792925 from (b) (4) mg/kg/day to 110 mg/kg/day was reasonable. However, the FDA stated that the proposal to (b) (4) was not acceptable.

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

4.1. Office of Scientific Investigations

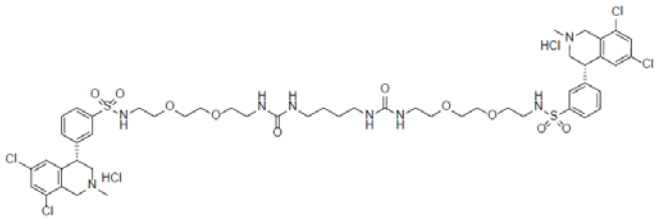
Five clinical investigator sites were selected for inspection, based on enrollment, prior inspection history, and number of open INDs in the Office of Scientific Investigations (OSI) database. Four of five received a final classification of No Action Indicated. One site received a final classification of Voluntary Action Indicated (site 180, Study 302, enrolled 19 subjects). The inspector concluded that while violations were noted at this site, they were not of a type or magnitude that are expected to have a significant impact on data reliability or on the rights, safety or welfare of subjects. Please refer to the finalized review by Dr. Susan Leibenhaut (April 5, 2019) for further details.

4.2. Product Quality

The active pharmaceutical ingredient in tenapanor tablets, 50 mg is tenapanor hydrochloride. It is a white to off-white to light brown amorphous solid. It can be designated as a Biopharmaceutical Classification System class IV compound due to its poor water solubility and permeability.

The active pharmaceutical ingredient is manufactured by (b) (4) The detailed chemistry, manufacturing, and controls (CMC) information regarding the manufacturing process, in-process controls, control of materials, critical steps, impurities and intermediates was provided. Its chemical structure was confirmed by modern spectroscopic methods (e.g., nuclear magnetic resonance, mass spectroscopy, and Fourier-transform infrared spectroscopy, as well as single crystal X-ray crystallography).

Figure 1. Structure of Tenapanor Hydrochloride



Molecular Formula: $C_{50}H_{68}Cl_6N_8O_{10}S_2$
Molecular Weight: 1217.97 (Free Base: 1145.04)

The overall quality of tenapanor hydrochloride is controlled by its specification. Based on the stability studies of multiple batches a retest period of (b) (4) months was granted.

Tenapanor tablets, 50 mg are white to off-white, oval, biconvex, (b) (4) immediate release tablets. The tablets are debossed with "50" on one side and "5791" on the other side. Each tablet contains 53.2 mg of tenapanor hydrochloride. The tablets also contain the following inactive ingredients: microcrystalline cellulose, low-substituted hydroxypropyl cellulose, colloidal silicone dioxide, tartaric acid, stearic acid as well as propyl gallate as (b) (4). The

tablets are supplied in white high-density polyethylene bottles. Each bottle contains 60 tablets and a silica gel desiccant.

The drug product is manufactured by (b) (4) The tablet manufacturing process includes (b) (4) The proposed commercial batch size is (b) (4) kg (approximately (b) (4) tablets). The overall control strategy for the drug product's identity, strength, purity and quality is deemed adequate based on raw material controls and drug product specification.

Based on satisfactory stability studies of the drug product, 24 months of expiration dating period is granted when stored at room temperature in the proposed container closure system.

The Applicant has provided sufficient CMC information to assure the identity, strength, purity and quality of the drug product. The Office of Process and Facilities has made an "Approval" recommendation for all manufacturing and testing facilities involved in this NDA.

The claim of a categorical exclusion from the requirements of an environmental assessment in accordance with 21 CFR Part 25.31(b) deemed acceptable.

The label/labeling is satisfactory from the CMC perspective.

Therefore, from the OPQ perspective, this NDA is recommended for approval with an expiration dating period of 24 months when stored at room temperature in the proposed container closure system.

4.3. Clinical Outcomes Assessment

A consultation from FDA clinical outcomes assessment staff (COA) was requested. The applicant initially proposed (b) (4)

COA staff were requested to assess the content validity to assess these concepts. They concluded that PRO items used to assess bowel frequency and abdominal pain are fit-for-purpose, (b) (4) because the sponsor did not have adequate qualitative evidence in support of the content validity. In addition, there were concerns that patients may not clearly and consistently understand concepts such as (b) (4)

(b) (4)

demonstrated on treatment. Therefore, these results were not included in product labeling. The reader is referred to the COA staff consultation review by Susan Pretko and Sarrit Kovacs, dated 7/23/19.

5. Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

Tenapanor is a locally acting sodium (Na+)/hydrogen (H+) exchanger 3 (NHE3) inhibitor. NHE3 is expressed on the apical surface of the small intestine and colon, and is primarily responsible for the absorption of dietary sodium. The Applicant is seeking approval of tenapanor for the treatment of irritable bowel syndrome with constipation (IBS-C).

In support of the safety of tenapanor, the Applicant submitted the following nonclinical studies: pharmacology, PK, single dose and repeat dose oral toxicity studies in rats (up to 6 months) and dogs (up to 9 months), a battery of genotoxicity studies, and reproductive and developmental toxicity studies. The Applicant also conducted carcinogenicity studies in two rodent species, and toxicity studies in juvenile animals with tenapanor.

Tenapanor is a potent inhibitor of rat and human NHE3 expressed in opossum kidney (OK) and Chinese hamster lung fibroblast (PS120) cells with IC_{50} values of 20 and 13nM, respectively. The major tenapanor metabolite, AZ13792925 (M1), was inactive against rat and human NHE3 with IC_{50} values of >10µM. Tenapanor did not inhibit human NHE1 and NHE2 activities at concentrations as high as 10µM in human embryonic kidney (HEK293) and OK cells transfected with human NHE1 and NHE2 genes, respectively. Oral administration of tenapanor to CD-1 mice (up to 15 mg/kg BID) and SD rats (up to 10 mg/kg) caused a dose-related increase in fecal water content, increased GI motility, increased fecal sodium and decreased urinary sodium levels. In cynomolgus monkeys, oral administration of tenapanor at 50 or 150 mg/kg caused soft feces and reduced urinary sodium. In a stress-induced IBS-C model of rat, and a mouse model of opioid-induced constipation, tenapanor showed a significant improvement of GI function following oral doses of up to 50 mg/kg. Oral administration of tenapanor (0.5 mg/kg) to a rat model of IBS-like syndrome (intracolonic infusion of 0.5% acetic acid) resulted in reductions in colonic hypersensitivity, visceral hypersensitivity and normalized colonic sensory neuronal excitability.

Tenapanor showed a slight inhibition (16.1%) of hERG current (IKr) at a concentration 10µM (highest concentration tested) in an in vitro assay in HEK293 cells. However, oral administration of tenapanor to conscious telemetered male beagle dogs at doses up to 1000 mg/kg had no effect on cardiac and circulatory functions and electrocardiogram (ECG) parameters. Rats receiving oral doses of tenapanor at doses up to 1000 mg/kg had no CNS adverse effects. AZ13792925 (major metabolite of tenapanor) showed no adverse effects in an in vitro human recombinant cardiac ion channel (hERG, $hK_v4.3$/hKChIP2.2 and $hK_v7.1$/hKCNE1) assay.

PK studies of tenapanor were conducted in rats and dogs. The bioavailability of tenapanor was very low in rats and dogs. Tenapanor was highly bound to plasma proteins of human, rats and dogs. In vitro studies showed that tenapanor was stable in simulated gastric and intestinal fluids for up to 6 days. Human, rat and dog microsomal metabolism studies with tenapanor identified more than five distinct metabolites. M1 (AZ13792925) was identified as major metabolite of

tenapanor. Studies in rats showed that more than 90% of tenapanor was excreted in the feces as the intact molecule.

Repeated dose toxicology studies of up to 6 months and 9 months were conducted in rats and dogs, respectively. In a 6-month toxicology study in rats, tenapanor was administered orally to SD rats at doses of 1, 3, and 10 mg/kg/day. Two males in the high dose group died on Days 65 and 139, respectively. Histological examinations of these animals showed hemorrhage and acute inflammation of the cecum; luminal dilatation of the duodenum, jejunum, ileum, and colon; and lymphoid depletion in the spleen and thymus. The unscheduled deaths of the two high dose group animals were related to gastrointestinal changes, and considered test article-related. Clinical observation in surviving animals included soft feces, diarrhea, and associated brown material around the anogenital and urogenital areas. The NOAEL was determined to be 3 mg/kg/day for males and 10 mg/kg/day for females. In a 9-month toxicology study in dogs, tenapanor was administered orally to beagle dogs at doses of 50, 300 and 1000 mg/kg/day. Test article-related clinical observations were limited to abnormal excreta, primarily soft feces and diarrhea in all tenapanor-treated male and female groups throughout the study. There were no test article-related mortalities, macroscopic, microscopic, or organ weight changes in dogs. The NOAEL was determined to be 1000 mg/kg/day.

Tenapanor was negative in the Ames test, the in vitro mammalian chromosome aberration assay in human peripheral blood lymphocytes, and the in vivo micronucleus assay in mice following oral and IV administration in rats. Tenapanor was not tumorigenic in 2-year rat and 6-month Tg rasH2 mouse carcinogenicity bioassays.

The potential effects of tenapanor on female fertility were assessed in CD-1 mice and the effects on male fertility were assessed in SD rats. There were no tenapanor-related effects on female or male fertility at doses up to 50 mg/kg/day in female mice and up to 10 mg/kg/day in male rats. In an embryofetal developmental study in rats, tenapanor was administered orally at dose levels of 1, 10 and 30 mg/kg/day from Gestation Days 6 through 17. Dose levels of 10 and 30 mg/kg/day were not tolerated by the pregnant rats and were associated with mortalities and moribundity, adverse clinical observations (primarily excreta-related), body weight loss, and reduced food consumption. Animals in these groups were sacrificed early on Gestation Days 13-16 without further examination. Intrauterine growth, survival and external, visceral, and skeletal morphology were unaffected in animals from the 1 mg/kg/day dose group. The NOAEL dose for maternal toxicity was <1 mg/kg/day, and the 1 mg/kg/day dose was considered to be the NOAEL for embryo/fetal development in rats. In an embryofetal development study in rabbits, tenapanor was administered orally at doses of 5, 15 and 45 mg/kg/day from Gestation Day 6 through 20. Mean body weight loss and reduced food consumption were noted at dose levels of 15 and 45 mg/kg/day. Based on these findings, the 5 mg/kg/day dose was considered to be the NOAEL for maternal toxicity, and the 45 mg/kg/day dose was considered to be NOAEL for embryofetal development in rabbits.

In a pre- and postnatal development study in mice, tenapanor had no treatment-related effects on the maternal animals, including gestation lengths, parturition or the mean number of implantation sites. Lower mean body weight gains were noted in the 60 and 200 mg/kg/day group F_1 male (up to 14.1%) and female (up to 17.3%) pups throughout the preweaning period

(Postnatal Day [PND] 1 to 21). Mean F_1 pup body weights at 20 mg/kg/day were not affected. The 20 mg/kg/day dose was considered to be the NOAEL for F_1 neonatal/developmental toxicity. The NOAEL for F_1 parental systemic and reproductive toxicity and F_2 neonatal/early postnatal toxicity was considered to be 200 mg/kg/day.

In a juvenile animal dose ranging study in rats, tenapanor was not tolerated (>20% decreased body weight gain) in male and female pups (PND 5) at dose levels of 5 and 10 mg/kg/day, leading to unscheduled termination of the study on Day 11 (PND 16). In a second dose range finding study in juvenile rats (PND 5), tenapanor was administered from PND 5 through PND 24 at doses of 0.1, 0.5, 2.5 or 5 mg/kg/day by oral gavage. There were mortalities in male and female pups at ≥0.5 mg/kg/day. Lower mean body weight gains were generally noted in the 5 mg/kg/day group males (up to 43.7%) and females (up to 35.2%). Slightly lower mean tibial lengths (4.9% to 11.4%) and microscopic bone changes were noted in the 0.5, 2.5, and 5 mg/kg/day group males and females on PND 25 which correlated with the lower body weight in these groups.

In an 8-week definitive juvenile toxicology study followed by a 4-week recovery period, tenapanor was administered to juvenile rats beginning at PND 5 at dose levels of 0.03, 0.1 and 0.3 mg/kg/day. In the high dose group, five males died on PND 11, 18, 19 and 45 and two females died on PND 17 and 24. In the 0.1 mg/kg/day dose group, one male and one female died on PND 57 and 58, respectively. This death was not considered test article-related because there were no adverse effects. Mean absolute body weights were lower up to 15.8% (males) and 16.8% (females) in the high dose group compared to the control group. Mean tibial lengths of the 0.3 mg/kg group from PND 22 (males) or 19 (females) were generally shorter than the control group. There were no test article-related organ weight, macroscopic or microscopic changes in any group. The NOAEL was determined to be 0.1 mg/kg/day, which does not provide a margin of safety to support study of pediatric patients <12 years of age. An additional juvenile toxicity study in juvenile rats starting at postnatal day 21 will be required (refer to Postmarketing Requirements and Commitment, Section 14) below.

AZ13792925 (M1) was identified as the major metabolite of tenapanor. In support of the safety of AZ13792925, the Applicant conducted the following studies with the metabolite: single dose toxicity and repeat-dose oral toxicity studies in a rodent up to 4 weeks, genotoxicity (Ames test and in vitro micronucleus assay), 6-month carcinogenicity study in Tg rasH2 mouse, and an embryo-fetal development study in rats. In a 28-day toxicology study, AZ13792925 was administered orally to hemizygous transgenic (Tg) rasH2 [CbyB6F1-Tg(HRAS)2Jic] mice. There were no test article-related adverse findings noted at up to 250 mg/kg/day.

AZ13792925 was negative in the Ames test and the in vitro micronucleus assay in L5178Y mouse lymphoma cells. AZ13792925 was not tumorigenic in the 6-month Tg rasH2 mice carcinogenicity bioassay. In an embryofetal development study of AZ13792925, in SD rats, dose levels of 30, 150, and 400 mg/kg/day were administered to pregnant rats. One female in the 400 mg/kg/day group was euthanized *in extremis* on Gestation Day 17 following marked body weight loss and reduced food consumption and clinical observations of pale body and gasping on the day of euthanasia. Adverse fetal findings were observed at the 400 mg/kg/day, which

may be related to maternal toxicity of the compound. The 150 mg/kg/day dose was considered to be the NOAEL for both maternal toxicity and embryo/fetal development.

In summary, repeated dose toxicology studies of up to 6 months and 9 months were conducted in rats and dogs, respectively. In a 6-month toxicology study in rats, tenapanor was administered orally to SD rats at doses of 1, 3, and 10 mg/kg/day. Two males in the high dose group died on Days 65 and 139, respectively. Histological examinations of these animals showed hemorrhage and acute inflammation of the cecum; luminal dilatation of the duodenum, jejunum, ileum, and colon; and lymphoid depletion in the spleen and thymus. The unscheduled deaths of the two high dose group animals were related to gastrointestinal changes, and considered test article-related. Clinical observation in surviving animals included soft feces, diarrhea, and associated brown material around the anogenital and urogenital areas. These adverse gastrointestinal effects in rats are expected pharmacological effects of tenapanor. The NOAEL was determined to be 3 mg/kg/day for males and 10 mg/kg/day for females. The NOAEL doses in male and female rats are 1.8 times and 5.9 times the recommended daily clinical dose of 100 mg (1,7 mg/kg/day). In a 9-month toxicology study in dogs, tenapanor was administered orally to beagle dogs at doses of 50, 300 and 1000 mg/kg/day. Test article-related clinical observations were limited to abnormal excreta, primarily soft feces and diarrhea in all tenapanor-treated male and female groups throughout the study. There were no test article-related mortalities, macroscopic, microscopic, or organ weight changes in dogs. The NOAEL was determined to be 1000 mg/kg/day. The NOAEL dose in dogs is 588 times higher than the recommended clinical dose of 100 mg/day. Thus, the toxicology studies in rats and dogs show that the rat is a more sensitive species for GI adverse effects of tenapanor as compared to the dog. The differences in the GI sensitivities between these two species can be explained on the findings of the higher levels of intestinal NHE3 expression in rats as compared to dogs. Thus, the nonclinical studies support the safety of the proposed clinical dose of tenapanor in the proposed clinical population.

5.2. Referenced NDAs, BLAs, DMFs

IND 108732: Tenapanor, for irritable bowel syndrome with constipation (IBS-C); Ardelyx, Inc.

5.3. Pharmacology

Study: In Vitro Evaluation of the Inhibition Potential of Tenapanor and Its Major Metabolite, AZ13792925, (pIC_{50}) on Rat NHE3, Human NHE3, Human NHE1 and Human NHE2 (Study # RDX5791-EF-01A; RDX5791-EF-02A; RDX5791-EF-04 and RDX5791-EF-12)

This study was conducted to determine the potency of tenapanor (RDX5791) and its metabolite (AZ13792925) against rat and human NHE3 and human NHE1 and NHE2 Na^+/H^+ antiport activity. The gene encoding rat and human NHE3 were transiently expressed in OK cells and hamster lung (PS120) cells, respectively. Genes encoding for human NHE1 and NHE2 were transiently expressed in OK and HEK293 cells, respectively. Cells were preincubated with 5µM 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF). Ethyl isopropyl amiloride (EIPA), a specific inhibitor of NHE1 with minimal activity against NHE3, was used to inhibit

endogenous NHE1 activity. The pIC_{50} value of tenapanor for rat and human NHE3 inhibition was 8.7 (IC_{50} =1 and 1.3nM, respectively) during preincubation. The pIC_{50} values of tenapanor for rat and human NHE3 inhibition at persistent assay were 7.7 and 7.9 (IC_{50} =20 and 13nM, respectively). The pIC_{50} value of tenapanor metabolite AZ13792925 during preincubation and persistent assay were >5.0 (IC_{50} ≥10µM). Tenapanor did not inhibit human NHE1 and NHE2 activity at concentrations up to 10µM in HEK293, and OK cells transiently transfected with the human NHE1 and NHE2 genes, respectively, with pIC_{50} of >5.

In conclusion, this study showed that tenapanor is a potent inhibitor of rat and human NHE3 activity expressed in OK and PS120 cells with IC_{50} values of 20 and 13nM, respectively. The tenapanor metabolite (AZ13792925) was inactive against rat and human NHE3 with IC_{50} of >10µM.

Study: The Effect of a Single Dose of RDX5791 on Fecal Water and Sodium Content in Normal Rats (Study # RDX5791-EF-03)

RDX5791 was administered orally to 8-week-old SD rats at dose levels of 0, 0.3, 1.0, or 3.0 mg/kg. Sixteen hours postdose urine and fecal samples were collected to determine fecal water content and sodium levels in urine fecal samples. A dose-dependent increase in fecal water content was observed. Additionally, fecal sodium levels increased, and urine sodium levels decreased significantly in the treatment groups.

Study: The Pharmacodynamic Effect of Tenapanor in the Mouse (Study # 1516 (b) (4))

Tenapanor was administered orally to male CD-1 male mice twice daily at dose levels of 0, 1.5, 5, or 15 mg/kg/day for 7 days, and urinary and fecal sodium level was measured from Days 1 through 7. A dose-dependent reduction of urinary sodium excretion was noted. This was accompanied by a significant increase in fecal sodium excretion on treatment Day 7. The results indicate that tenapanor effectively inhibits intestinal sodium absorption in mice.

Study: The Effect of RDX5791 on Gastrointestinal Transit in Sprague-Dawley Rats (Study # RDX5791-EF-01-01)

Seven-week old SD rats (n=6) were administered RDX5791 (10 mg/kg) or the vehicle orally following 16 hours of overnight fasting. A 12% (weight/volume) carmine red dye was also administered orally immediately after test article administration, and the rats were necropsied 1 and 6 hours after dosing to determine the length of the intestine and the distance travelled by the red dye. The results showed that in RDX5791 treated rats, the dye traversed an average of 99.5% GI distance, demonstrating an increased transit rate.

Oral administration of tenapanor to mice, cynomolgus monkeys, a stress-induced IBS-C model of rat, and a mouse model of opioid-induced constipation showed a significant improvement in GI function. See nonclinical pharmacology/toxicology appendix (Section 16.2) for details of the studies.

5.3.1. Secondary Pharmacology

Study: AZD1722 and AZ13792925: Selectivity Screening in Radioligand Binding, Enzyme Activity and Functional Assays In Vitro (Study # 3126SV and 3534SV)

In a receptor screening assay, 181 and 82 distinct molecular targets (receptors, ion channels, transporters and enzymes) were screened for tenapanor and its metabolite (AZ13792925) bindings, respectively. The binding activity was determined with concentration-response curves generated where >25% activity was detected. The binding activity was defined as IC_{50} or EC_{50}. Tenapanor binding was observed for 41 targets but only two targets were identified with functional IC_{50} values of <1µM (5-HT1D receptor, 0.070µM and tachykinin NK1 receptor, 0.35µM). However, human plasma exposures are typically below the quantitative limit (<0.5 ng/mL) at steady state. Thus, these off-target activities are not expected to have any clinical relevance at therapeutic doses of tenapanor. Similarly, AZ13792925 (major metabolite of tenapanor) had four targets with IC_{50} at <1µM. They are the dopamine D_3 receptor (0.13µM), 5-HT7 receptor (0.24µM), 5-HT2B receptor (0.37µM), and carbonic anhydrase II (0.43µM).

5.3.2. Safety Pharmacology

Study: In Vitro Effect on hERG Current (IKr) Expressed in Human Embryonic Kidney Cells (Study # RDX5791-SP-01)

RDX5791 was tested at concentrations of 1, 3, and 10µM on hERG current in HEK293 cells. Whole cell patch clamp was used to study potassium current in HEK293 cells stably expressing the hERG channel to determine the effect of extracellular application of RDX5791 on the hERG current amplitude. Peak current values from each experiment were determined from the average of five consecutive experiments in each treatment period. The tail peak hERG current was reduced by 90% when cells were treated with positive control (E-4031) at 100nM, confirming cell sensitivity. RDX5791 at 1µM had no effect (0%) on the hERG currents but at 3µM and 10µM caused slight inhibitions of hERG currents by 5% and 16.1%, respectively. The clinical exposures in humans at 50 mg BID were <0.5 ng/mL. The, 10µM concentration is >24300-fold higher than the clinical exposures at the proposed dose.

Study: AZ13667691: Selectivity Screening in Cardiac Ion Channel Electrophysiological Assays In Vitro (Study # 3624SV)

The purpose of this study was to perform in vitro electrophysiological assays to assess the pharmacological activity of AZ13667691 (tenapanor) in a panel of human recombinant cardiac ion channels. Seven types of human recombinant voltage-gated cardiac ion channels were selected. They are $hCa_v1.2/\beta2/\alpha2\delta$ ($hICa_L$), $hCa_v3.2$ (ICa_T), hHCN4 (I_F), hKv1.5 (IK_{UR}), $hK_v4.3$/hKChIP2.2 (I_{to}), $hK_v7.1$/hKCNE1 (IK_s) and $hNa_v1.5$(INa). Electrophysiological assays were conducted in concentration-response mode up to a highest concentration of 33 or 100µM. The result showed that tenapanor had significant activity at one of the seven human recombinant voltage-gated cardiac ion channels ($hCa_v1.2/\beta2/\alpha2\delta$ ($hICa_L$) with IC_{50} value of 4.67 at 33.3µM of tenapanor. The 4.67 µM concentration is several thousand fold higher than the clinical exposure at the recommended dose, and thus, does not raise safety concerns.

Study: Evaluation of Cardiovascular Function Following Administration of RDX5791 in Conscious Telemetered Male Beagle Dogs (Study # RDX5791-SP-05)

The objective of this study was to determine the potential effects of RDX5791 on the cardiovascular system in conscious male beagle dogs, using a telemetry system. Blood pressure (systolic, diastolic, and mean arterial pressure), heart rate, ECG parameters (PR interval, QRS duration, RR interval, QT interval), and QTc were recorded by a transplanted transducer in four male beagle dogs/dose group. RDX5791 (100, 300 and 1000 mg/kg) or vehicle was administered orally on Days 1, 4, 8, and 11 at escalating doses. Data were collected continuously for 22 hours. Data were analyzed starting at 15 to 20 minutes before dosing and 0.5, 1, 2, 4, 8, 12 and 22 hours after dosing. A washout period of at least 72 hours was applied between the doses. No test article-related changes in systolic, diastolic, or arterial pressure, heart rate, cardiac rhythm, and ECG were noted at doses up to 1000 mg/kg.

Rats receiving oral doses of tenapanor at doses up to 1000 mg/kg had no CNS and respiratory adverse effects. See the appendix (Section 16.2) for details of the studies.

5.4. ADME/PK

PK studies of tenapanor were conducted in rats and dogs. The bioavailability of tenapanor was very low in rats and dogs. Tenapanor was highly bound to plasma proteins of human, rats and dogs. See the appendix (Section 16.2) for details.

5.5. Toxicology

5.5.1. General Toxicology

Study Title/Number: A 6-Month Oral (Gavage) Toxicity Study of AZD1722 in Sprague Dawley Rats With Male Fertility Evaluation (Study # (b) (4) -775030)

Key Study Findings

- Two males in the high dose group (10 mg/kg/day) died on Days 65 and 139, respectively. The male which died on Day 65 had intestinal torsion. Gross necropsy observations include: distended duodenum, jejunum, ileum, cecum, and colon, dark red areas in the glandular stomach, and dark red discoloration of the cecum. Microscopic examinations of both animals showed hemorrhage and acute inflammation of the cecum and lymphoid depletion in the spleen and thymus. The death of these animals was considered to be test article-related.
- Test article-related lower body weights and body weight changes were noted in the 3 mg/kg/day (up to 6.7%) and 10 mg/kg/day (up to 11.5%) dose group males and 10 mg/kg/day dose group females (up to 8%). Test article-related clinical chemistry parameters including decreased glucose, increased phosphorus and increased potassium levels were observed in the high and mid dose males; increased phosphorus and potassium levels were noticed in low dose males. Females in the high dose group showed decreased glucose levels and in the high and mid dose females had increased phosphorus levels.

- The NOAEL was determined to be 3 mg/kg/day for males and 10 mg/kg/day for females.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 4: Methods of Study AZD1722 in Sprague Dawley Rats

Parameter	Details
Dose and frequency of dosing:	0, 1, 3, and 10 mg/kg/day (5 mL/kg), once daily for 6 months
Route of administration:	Oral gavage
Formulation/vehicle:	0.1% Tween 80 [w/v] in deionized water
Species/strain:	Crl:CD (Sprague Dawley) rat
Number/sex/group:	20 males and 15 females/dose group.
Age:	7 weeks
Satellite groups/unique design:	For TK studies, 3/sex in control and 9/sex in low, medium and high dose groups
Deviation from study protocol affecting interpretation of results:	None.

Abbreviations: w/v, weight/volume; TK, toxicokinetic

Table 5: Observations and Results: Changes From Control, Study AZD1722 in Sprague Dawley Rats

Parameters	Major findings
Mortality	Two males (#9102 and #9106) from the 10 mg/kg/day dose group died on Days 65 and 139 after dosing.
Clinical signs	Soft feces, diarrhea, and associated brown material around the anogenital and urogenital areas were noted in a dose-related manner in the 1, 3, and 10 mg/kg/day dose groups. These observations were expected pharmacological effects of the test article.
Body weights	Test article-related lower body weights and body weight changes were noted in the 3 mg/kg/day (up to 6.7%) and 10 mg/kg/day group males (up to 11.5%), and 10 mg/kg/day females (up to 8%). The effect on body weight did not persist through the end of the dosing period.
Ophthalmoscopy	No ocular changes were observed.
Hematology	No alteration in hematology and coagulation parameters.
Clinical chemistry	HD males at Weeks 13 and 26: 8.0 and 9.8% decrease in mean glucose; 13.5% and 14.7% increase in phosphorus; 5.7% and 11.8% increase in potassium. MD males at Weeks 13 and 26: 5.3 and 11.5% decrease in mean glucose; 5.5% and 11.3% increase in phosphorus; 5.1% and 8.7% increase in potassium. LD males at Weeks 13 and 26: 4.1% and 11.3% increase in phosphorus; 6.4% and 14% increase in potassium HD females at Week 13: 11.5% decrease in mean glucose; 14.1% increase in phosphorus MD females at Week 13: 12.5% increase in phosphorus LD female at Week 13: 9.4% increase in phosphorus

Parameters	Major findings
Urinalysis	At Weeks 13 and 26, dark, yellow or red urine was observed in HD, MD and LD males and HD and MD females.
Gross pathology	No treatment-related macroscopic findings.
Organ weights	Relative to body weight (%) No test article-related effects in organ weights.
Histopathology Adequate battery: Yes	Hypertrophy of the zona glomerulosa (most superficial layer of the adrenal cortex): Male: LD: 15/15 (minimal 7, mild 7 and moderate 1) MD: 15/15 (mild 6 and moderate 9) HD: 14/14 (mild 6 and moderate 9) Female: LD: 15/14 (minimal 3, mild 11 and moderate 0) MD: 15/15 (mild 5 and moderate 10) HD: 15/15 (mild 5 and moderate 10) Mammary gland adenocarcinoma Female: HD: 1/15 (This finding is not considered test article-related due to lack of any potential precursor lesions, and considered to be back ground finding in rats).
[Other evaluations] Male fertility evaluation	Described under reproductive and developmental toxicology, Section 16.2.

-: indicates reduction in parameters compared to control.
Abbreviations: LD, low dose; MD, middle dose; HD, high dose

Study Title/Number: A 9-Month Oral (Capsule) Toxicity Study of AZD1722 in Beagle Dogs (Study # (b) (4) -775031)

Key Study Findings

- One male (#3296) in the low dose group (50 mg/kg/day) was euthanized *in extremis* prior to scheduled necropsy on study day 208. This dog had a stricture of the jejunum due to entanglement with a vas deferens. This death was not considered a treatment related finding because only one low dose animal was affected.
- There were no test article-related effects on body weight, food consumption, hematology, coagulation, urinalysis, organ weight, ophthalmic, electrocardiographic, macroscopic or microscopic findings.
- The NOAEL was 1000 mg/kg/day, the highest dosage tested for both males and females.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 6: Methods of Study of AZD1722 in Beagle Dogs

Parameter	Details
Dose and frequency of dosing:	0, 50, 300 and 1000 mg/kg/day, once daily
Route of administration:	Orally
Formulation/vehicle:	Size 11, gelatin capsules
Species/strain:	Beagle dogs
Number/sex/group:	4 animals/sex/dose groups
Age:	5 to 6 months
Satellite groups/unique design:	None
Deviation from study protocol affecting interpretation of results:	None.

Table 7: Observations and Results: Changes From Control, Study AZD1722 in Beagle Dogs

Parameters	Major Findings
Mortality	One male (#3296) in the low dose group (50 mg/kg/day) was euthanized *in extremis* prior to scheduled necropsy on study day 208, and was not considered treatment-related.
Clinical signs	Test article-related clinical observations were limited to abnormal excreta, primarily soft feces and diarrhea in all AZD1722-treated male and female groups throughout the study. These observations were expected pharmacological effects of the drug.
Body weights	No test article-related effects on body weights.
Ophthalmoscopy	No ocular changes were observed.
Electrocardiogram	No test article-related effects were observed. However, the mean QRS interval in the 1000 mg/kg/day group males (38 msec) was significantly decreased compared to the control group (44 msec) during study Week 38 at approximately 6 hours postdosing. This statistically significantly minor change in the QRS interval was a normal variant, as values were still within the normal range.
Hematology	No alteration in hematology and coagulation parameters.
Clinical chemistry	No test article-related differences in clinical chemistry parameters
Urinalysis	No test article-related alterations in urinalysis parameters
Gross pathology	No treatment-related macroscopic findings.
Organ weights	Relative to body weight (%) No test article-related effects in organ weights.
Histopathology Adequate battery: Yes	No test article-related histopathological findings were noted.
[Other evaluations]	None

-: indicates reduction in parameters compared to control.
LD = low dose; MD = middle dose; HD = high dose

Twenty-eight day and 3-month oral gavage toxicity studies of tenapanor were conducted in SD rats. In a 28-day dose range finding study in rats, tenapanor was administered up to 100 mg/kg/day. In this study, there was high mortality and severe adverse effects on body weight, and the study was shortened from 4 weeks to 2 weeks. In a 3-month toxicology study in rats, the NOAEL was 5 mg/kg/day (highest dose tested). In a 3-month oral gavage toxicity study in dogs, the NOAEL was determined to be 1000 mg/kg/day (highest dose tested). See the appendix (Section 16.2) for details.

5.5.2. Genetic Toxicology

Tenapanor was negative in the Ames test, the in vitro mammalian chromosome aberration assay in human peripheral blood lymphocytes, and the in vivo micronucleus assay in mice following oral and IV administration in rats. See the appendix (Section 16.2) for details.

5.5.3. Carcinogenicity

Study: AZD1722: A 104-week Carcinogenicity Study by Oral Gavage in Rats (Study # 3732CR [20049785]

In a 2-year rat carcinogenicity study, tenapanor was administered to Crl:CD (Sprague Dawley) rats by oral gavage at dose levels of 0 (vehicle), 1, 3, and 10 mg/kg/day. The high dose of 10 mg/kg/day was selected based on the MTD from the 14-day and 6-month toxicology studies in rats. On Day 86, the 10 mg/kg/day dose level was reduced to 5 mg/kg/day due to a decrease in body weight up to 20%. On Day 92, the 3 mg/kg/day (mid dose) dose level was reduced to 2 mg/kg/day for both male and female rats. Males in the high dose group (10/5 mg/kg/day) were terminated early at Week 83, as only 15 animals survived in the group. All other male groups were terminated at Week 86 after only 20 animals survived in the male vehicle control group. All female groups were terminated on Week 96 due to survival in the vehicle control female group reached 20 animals. There were no tenapanor-related significant effects on survival of male or female rats. Tenapanor treated animals showed up to 20% reduction in body weight within the first 3 months of the study. Treatment with tenapanor was not associated with a treatment related significant increase in neoplasms in male and female rats.

Study: A 26-week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1/Tg rasH2 hemizygous mice (Study No. RDX5791-TX-16 [20105943])

In the 26-week carcinogenicity study in Tg rasH2 mice with tenapanor and its major metabolite, AZ13792915, tenapanor doses of 100, 300 and 800 mg/kg/day were used for females, and 10, 30 and 100 mg/kg/day for males. The doses of AZ13792915 used were 55 and 165 mg/kg/day for both male and female mice. The control groups received the vehicles. The positive control was 7.5 mg/mL N-nitrosomethylurea in citrate-buffered saline (pH 4.5). There were no tenapanor or AZ13792925-related effects on survival rates of male or female Tg rasH2 mice. For the positive control group, there was a high incidence of mortality in males (13 out of 15) and females (12 out of 15). Males and females in the 165 mg/kg/day AZ13792925 dose group showed reductions of body weight (13.3% and 8.3%, respectively), compared to controls. Due to decrease in mean body weight, high dose of 165 mg/kg was reduced to 110 mg/kg/day on Day 99. The body weight was lower in males (12.8%) and females (7.0%) at study termination day. No significant tenapanor or AZ13792925-related neoplastic or non-neoplastic findings were noted in male and female Tg rasH2 mice.

5.5.4. Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Female fertility and early embryonic development study was conducted in CD-1 mice. The NOAEL for female fertility was determined to be 50 mg/kg/day (highest dose tested). Effects of

tenapanor in male fertility was determined in SD rats. The NOAEL was determined to be 10 mg/kg/day (highest dose tested). See the appendix (Section 16.2) for details.

Embryo-Fetal Development

Study Title/Number: An Oral (Gavage) Study of Effects of RDX5791 on Embryo/Fetal Development in Rats (Study # RDX5791-TX-14 [(b) (4)-775024])

Key Study Findings

- Oral gavage administration of tenapanor to pregnant Crl:CD (Sprague Dawley) rats during organogenesis was not well-tolerated at dose levels of 10 and 30 mg/kg/day, as evidenced by mortality and moribundity, adverse clinical findings (primarily excreta-related), body weight losses, and reduced food consumption. Due to excessive maternal toxicity, females in the 10 and 30 mg/kg/day group were terminated early on Gestation Days 13, 14, 15, or 16.
- In the 1 mg/kg/day group of tenapanor, excreta related clinical findings were noted generally throughout the treatment period. Intrauterine growth, and survival and external, visceral, and skeletal morphology were unaffected in the 1 mg/kg/day. Mean body weight losses and reduced food consumption were noted at 1 mg/kg/day.
- The NOAEL for maternal toxicity was determined to be <1 mg/kg/day, and NOAEL for the embryo-fetal development was considered to be 1 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 8: Methods of Study RDX5791-TX-14 on Embryo/Fetal Development in Rats

Parameter	Details
Dose and frequency of dosing:	0, 1, 10 and 30 mg/kg/day, once daily.
Route of administration:	Oral gavage
Formulation/vehicle:	0.1% Tween 80 [w/v] in deionized water
Species/strain:	Crl:CD (Sprague Dawley) rats
Number/sex/group:	25 females/dose group
Satellite groups:	Yes, for toxicokinetic analysis five females/dose group
Study design:	Pregnant animals were dosed once daily by oral gavage, from GD 6 to GD 17. Females were observed daily for clinical signs, and body weights and food intake was recorded daily. On GD 20, a laparohysterectomy was performed on each surviving female. The uteri, placentae, and ovaries were examined, and the number of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations. For toxicokinetic analysis, blood samples were collected from four rats/test article treated group at 0 (predose), 1, 2, 4, 8, and 24 hours after dose administration on GDs 6 and 17.
Deviation from study protocol affecting interpretation of results:	None

Abbreviation: GD, gestation day

Table 9: Observations and Results of Study RDX5791-TX-14 on Embryo/Fetal Development in Rats

Parameters	Major findings
Mortality	In the 30 mg/kg/day dose group, two females were found dead (#32879 and 32859) on GD 13 and 16, respectively, and one female (#32862) was euthanized *in extremis* on GD 15. Mortality was not observed in the 10 mg/kg dose group. However, due to extreme body weight loss and significant reduction in food intake, females in the MD and HD groups were terminated on GDs 13, 14, 15, or 16. All females in the control and 1 mg/kg/day groups survived to the scheduled necropsy on GD 20.
Clinical signs	Females in the 10 and 30 mg/kg/day dose group showed soft feces and diarrhea, decreased defecation, and brown material on the anogenital area, urogenital area, base of the tail, and ventral abdominal area. These findings were noted at the daily examinations generally beginning on GD 7 through the day of death/euthanasia. In addition, red material on the urogenital area was noted for two and six females in the 10 and 30 mg/kg/day groups, respectively, during GDs 13-16 at the daily examinations and/or 1-2 hours following dose administration. Partial closure of the eyes was noted on GD 13 at the daily examinations for the female that was euthanized *in extremis* in the 30 mg/kg/day group. Clinical findings of soft stool/diarrhea were noted for all females in the 1 mg/kg/day group, generally throughout the treatment period.
Body weights	Animals in all MD and HD groups showed statistically significant decrease in body weight gain (MD: up to 28.6%; HD: up to 35%,) on Day 6 to 16 of gestation when compared to controls. In the LD group, mean body weights were 5% to 6.8% lower compared to the control group.
Necropsy findings Cesarean section data	Two females found dead (#32879 and 32859) in the HD group showed small thymus. One female euthanized *in extremis* showed distended cecum with 14 normally developed implantations and one early resorption in utero. Due to extreme body weight loss and significant reduction in food intake, females in the MD and HD groups were terminated on GDs 13, 14, 15, or 16. These females from the MD and HD were discarded without examination. Data below shows from control and low dose groups only. No treatment-related findings in LD group (1 mg/kg/day) at necropsy in any female sacrificed on Day 20 of gestation. *(continued below)*

Parameters	Major findings
Necropsy findings Cesarean section data *(continued)*	Corpora lutea, number CG: 420±3.15 LD: 425±2.74 Implantations, number CG: 390±2.6 LD: 382±3.55 Early Resorption (%) CG: 4.8% LD: 6.0% Late Resorption (%) CG: 0.00% LD: 4.00% Pre-implantation loss (%) CG: 6.2% LD: 11.0% Post implantation loss (%) CG: 4.8% LD: 10.0% Fetal Weight (g) CG: 3.7 LD: 3.6
Necropsy findings Offspring	All animals in the 10 and 30 mg/kg/day groups were found dead, euthanized in extremis, or euthanized early due to excessive toxicity prior to the scheduled necropsy. Therefore, evaluation of fetal morphology was precluded in these groups. There were no test article-related skeletal developmental variations noted in the 1 mg/kg/day group. An increased mean litter proportion of reduced ossification of the vertebral arches was noted in the 1 mg/kg/day group (1.7% per litter) compared to the control group (0.0% per litter) and the value exceeded the maximum mean value in the WIL historical control data (1.1% per litter).

Abbreviations: CG, control group; GD, gestation day; HD, high dose; LD, low dose; MD, middle dose

Study Title/Number: An Oral (Gavage) Study of the Effects of RDX5791 on Embryo-Fetal Development in Rabbit (Study # RDX5791-TX-15 [(b) (4)-775025])

Key Study Findings

- Females in the MD and HD dose groups showed mean body weight loss and reduced food intake.
- Tenapanor had no effect on the pregnancy or fetal parameters at any dose levels.
- The NOAEL for maternal toxicity was determined to be 5 mg/kg/day due to weight loss, and embryo-fetal development was considered to be 45 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 10: Methods of Study RDX5791-TX-15 on Embryo-Fetal Development in Rabbit

Parameter	Details
Dose and frequency of dosing:	0, 5, 15 and 45 mg/kg/day, once daily
Route of administration:	Oral gavage
Formulation/vehicle:	0.1% Tween 80 [w/v] in deionized water
Species/strain:	New Zealand white rabbits
Number/sex/group:	22 females/dose group
Satellite groups:	4 females/dose group for TK analysis. Blood samples were collected on GDs 7 and 20.
Study design:	Pregnant females were dosed once daily by oral gavage, from GD 7 to GD 20. Females were observed daily, their body weights were recorded, and food intakes monitored. Animals were sacrificed on Day 29 of gestation and pregnancy parameters were determined. Gravid uterus and placenta weights were recorded. Fetuses were removed from the uterus, weighed, sexes determined and then examined for external, visceral and skeletal abnormities.
Deviation from study protocol affecting interpretation of results:	None.

Abbreviation: w/v, weight/volume; GD, gestation day; TK, toxicokinetic

Table 11. Observations and Results of Study RDX5791-TX-15 on Embryo-Fetal Development in Rabbit

Parameters	Major findings
Mortality	No test article-related mortality.
Clinical Signs	One female in the 45 mg/kg/day group aborted on GD 23 following body weight losses (17.8%) and reduced food consumption beginning 7 days prior to abortion. This single abortion was not considered test article-related. Slightly increased incidences of excreta-related findings (decreased defecation, small feces, soft stool, diarrhea, and/or brown material at the base of the tail) were noted in all test article treated groups at the daily examinations beginning on GD 8. These excreta related findings were generally noted in a dose-dependent manner and were expected pharmacological effects of the test article. No other test article-related clinical signs were noticed.
Body Weights	There was a reduction in overall mean body weight gain (g) in MD and HD females (LD: 240±41.5g; MD: 82±62g; HD: 138±40.4g vs. 320±33.1g in controls) during the treatment period (Days 7 to 21 of gestation) compared with controls; (statistically significant at 15 and 45 mg/kg/day; $p<0.01$ to $p<0.05$). After the dosing was stopped, body weight gain was similar in all groups on Day 29 of gestation.

Parameters	Major findings
Necropsy findings Cesarean section data	One female in the 45 mg/kg/day dose group (#65633) aborted on GD 23, and had white areas on a thickened cecum and colon and two early resorptions in utero. No other treatment-related findings were observed at necropsy. Treatment with tenapanor had no significant effect on the pregnancy parameters at any dose levels. Corpora lutea, number CG: 215±1.6 LD: 208±1.9 MD: 188±1.9 HD: 195±2.1 Implantations, number CG: 189±1.2 LD: 196±1.6 MD: 172±2.3 HD: 178±1.6 Early Resorption (%) CG: 3.2 LD: 10.4 MD: 3.2 HD: 6.0 Late Resorption (%) CG: 0.5 LD: 2.9 MD: 3.8 HD: 2.8 Pre-implantation loss (%) CG: 11.1 LD: 4.9 MD: 9.0 HD: 7.9 Post implantation loss (%) CG: 3.8 LD: 13.3 MD: 7.0 HD: 8.7 Fetal Weight (g) CG: 40.5 LD: 39.0 MD: 39.8 HD: 40.1

Parameters	Major findings
Necropsy findings Offspring malformations, variations, etc.	Tenapanor treatment was not associated with any significant increase in fetal abnormalities at any dose level. However, some malformations were observed which include: CG: One fetus with excessive fat pad. LD: Four fetuses were noted with lobular agenesis of the lungs consisting of an absent right accessory lobe. Fusion of sternebrae numbers 4 and 5. Vertebral central anomaly in one fetus. MD: One fetus with hydrocephaly. HD: One fetus with carpel flexure (right). Another fetus (#65656-06) had an absent kidney and ureter (right). Fetus no. 65625-09 was noted with a skull anomaly consisting of the intraparietal and supraoccipital bones being fused. Fusion of sternebrae nos. 1 through 5. However, none of these findings were considered treatment-related because of isolated cases of these findings and the incidences are within historical control values.

Abbreviations: CG, control group; LD, low dose; MD, mid dose; HD, high dose; GD, gestation day

Prenatal and Postnatal Development

Study Title/Number: An Oral (Gavage) Study of the Effects of Tenapanor on Pre- and Postnatal Development, Including Maternal Function in Mice (Study # (b) (4) -775035)

Key Study Findings

- Tenapanor had no effect on pregnancy parameters or litter survival, percentage of males at birth, postnatal survival, pup clinical observations, or necropsy findings at any dosage level.
- Test article-related, adverse lower mean body weight gains were noted in the 60 mg/kg/day (8.3% to 14.1%) and 200 mg/kg/day (13.7% to 17.3%) group F_1 male and female pups throughout the preweaning period (PND 1 to 21).
- Maternal treatment of tenapanor had no other significant effect on the F_1 generation, including behavior, sexual development, and fertility and mating performance in either sex.
- The NOAEL for maternal toxicity was determined to be 200 mg/kg/day and the NOAEL for the F_1 neonatal/developmental toxicity was considered to be 20 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 12: Methods of Study (b) (4)-775035 on Pre- and Postnatal Development and Maternal Function in Mice

Parameter	Details
Dose and frequency of dosing:	0, 20, 60 and 200 mg/kg/day; Once daily
Route of administration:	Oral gavage
Formulation/vehicle:	Tween 80 [w/v] in deionized water
Species/strain:	Crl:CD1 Mice
Number/sex/group:	25 females/dose group
Satellite groups:	None
Study design:	Animals were dosed orally once daily, from GD 6 to Lactation Day 20. Females were observed daily for clinical signs, and body weights and food intake was also recorded daily. The females were allowed to litter and rear their offspring to weaning on Day 21 of lactation. Pups not selected for the F_1 generation were sacrificed on Day 21 of lactation together with all parental generation dams, and a necropsy examination was conducted. For the F_1 generation, 25 males and 25 females per group were selected and allowed to mature, untreated. The effects on growth, development, behavior and reproductive performance were assessed. The F_1 animals in each group (maximum of 1/sex/litter) were mated, avoiding sibling pairings. All F_1 females were allowed to deliver and rear their pups until PND 4. Clinical observations, body weights, and sexes were recorded for F_2 pups at appropriate intervals. Gross necropsies were performed on all F_2 pups found dead; all remaining F_2 pups were euthanized and discarded without examination on PND 4.
Deviation from study protocol affecting interpretation of results:	None.

Abbreviations: PND, postnatal day; w/v, weight/volume; GD, gestation day

Table 13: Observations and Results of Study (b) (4)-775035 on Pre- and Postnatal Development and Maternal Function in Mice

Generation	Major Findings
F0 dams	There were no test article-related effects on survival of the F_0 females at any dosage level. One female in the 200 mg/kg/day group was found dead on GD 8. In the 20 mg/kg/day group, one female was euthanized *in extremis* on lactation Day 3. The moribundity and mortality at 20 and 200 mg/kg/day occurred in the absence of any other signs of adverse toxicity, and therefore were not considered test article-related. No test article-related clinical signs and body weight gains were noted during the study. Lower mean food consumption was noted in the 200 mg/kg/day group F_0 females throughout the entire gestation (GDs 6 to 18) and lactation (lactation days 1-21) periods. However, in the absence of an effect on mean body weights, the reduction in mean food consumption was considered test article-related but not adverse. There were no test article-related effects on gestation lengths or the process of parturition for F_0 females at any dosage level. There were no test article-related macroscopic findings or effects on the mean number of implantation sites in any dose group.
F1 generation	No unscheduled deaths or clinical signs related to the test item were observed in either sex. There were no test article-related effects on the mean number of F_1 pups born, live litter size, percentage of males at birth, postnatal survival, pup clinical observations, or necropsy findings at any dosage level. Test article-related, adverse lower mean body weight gains were noted in the 60 mg/kg/day (8.3% to 14.1%) and 200 mg/kg/day (13.7% to 17.3%) group F_1 male and female pups throughout the preweaning period (PND 1-21). There were no effects of maternal treatment on learning and memory of F1 pups. However, a test article-related higher (up to 64.4%) mean escape time with a corresponding higher (up to 110.0%) mean number of errors was noted in the 200 mg/kg/day group F_1 males during the PND 22 memory assessment. There were no effects of maternal treatment on auditory function, sexual development, fertility and mating performance in either sex at any dose levels. However, test article-related higher motor activity counts were noted for F_1 males at 200 mg/kg/day on PND 21 but not in F_1 males and females at 20 and 60 mg/kg/day or F_1 females at 200 mg/kg/day on PND 21 and or at any dosage level on PND 61.
F2 generation	No effects of F_0 maternal test article administration were noted on the mean number of F_2 pups born, live litter size, percentage of males at birth, postnatal survival or pup clinical observations.

Abbreviation: GD, gestation day; PND, postnatal day

5.5.5. Juvenile Toxicology Studies

In the 8-week juvenile toxicology study in rats, mortalities and dehydration of the animals were observed and the NOAEL was determined to be 0.1 mg/kg/day. Details of the studies are provided in the appendix (Section 16.2).

These findings of toxicity at doses much lower than human therapeutic doses raise concerns of the potential for young children to have increased susceptibility to toxicity related to tenapanor. The findings in this study occurred in animals of human age equivalent < 2 years.

While IBS does not occur in this young age group (age < 6 years), there is a need to study the drug in children age 6 years and older. This issue will be further evaluated in an additional juvenile toxicology study, issued as a post-marketing requirement, with dosing to start on PND21, to determine whether or not it is appropriate to initiate any clinical trial in patients <12 years of age. The currently available information will be noted in product labeling, which will include a boxed warning noting the deaths in young juvenile animals, a contraindication in patients < 6 years, and a warning to avoid use in patients 6 to <12 years of age.

6. Clinical Pharmacology

6.1. Executive Summary

IBSRELA (tenapanor) is a locally acting sodium/hydrogen exchanger 3 (NHE3) inhibitor. The proposed indication is for the treatment of irritable bowel syndrome with constipation (IBS-C) in adults. IBSRELA is formulated as a tablet containing 50 mg of tenapanor. The proposed dosing regimen is 50 mg administered orally twice a day (50 mg BID), immediately prior to breakfast (or the first meal of the day) and immediately prior to dinner.

The Applicant evaluated the 50 mg BID dosing regimen in two phase 3 trials (TEN-01-301 and TEN-01-302). The Applicant conducted a phase 2 dose-ranging study to support the dose selection for phase 3 trials. The Applicant also submitted results of phase 1 trials and in vitro studies to support clinical pharmacology information of tenapanor.

The key clinical pharmacology review findings are summarized in Table 14.

Table 14. Summary of Clinical Pharmacology Review Findings

Review Issues	Recommendations and Comments
Pivotal and supportive evidence of effectiveness	The efficacy of tenapanor for the treatment of IBS-C is established in two phase 3 trials (TEN-01-301 and TEN-01-302). The pharmacodynamic effect on fecal sodium excretion in phase 1 trial (D5611C00005) and dose-response for complete spontaneous bowel movement (CSBM) in phase 2 trials (RDX5791-201 and D5612C0001) provide supportive evidence of effectiveness.
General dosing instructions	The efficacy and safety data from phase 3 trials overall support that the proposed 50 mg BID dosing regimen is acceptable.
Dosing in patient subgroups (intrinsic factors)	No dose individualization is recommended based on intrinsic factors.
Drug interactions	In vitro and in vivo drug interaction studies do not indicate clinically relevant drug-drug interaction (DDI) potential for tenapanor.
Bridge between the to-be marketed and clinical trial formulations	The to-be-marketed formulation was used in tenapanor phase 3 trials; therefore, there is no need to bridge the to-be-marketed formulation to the clinical trial formulation.

Abbreviation: IBS-C, irritable bowel syndrome with constipation

6.1.1. Recommendations

From a clinical pharmacology standpoint, this NDA is acceptable to support the approval of tenapanor for the treatment of irritable bowel syndrome with constipation in adults.

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Mechanism of Action

Tenapanor is a locally acting inhibitor of the sodium/hydrogen exchanger 3 (NHE3). NHE3 is an antiporter expressed on the apical surface of the small intestine and colon primarily responsible

for the absorption of dietary sodium. In vitro and animal studies indicate that the major metabolite of tenapanor, M1 (AZ13792925), is not active against NHE3.

Pharmacodynamics

Tenapanor reduces absorption of sodium from small intestine and colon. In a study in healthy subjects, taking tenapanor (tablet 15 mg) 5 to 10 minutes before a meal showed a greater effect on increasing the 24-hour stool sodium excretion than taking tenapanor in fed or fasting conditions.

QT Prolongation

At approximately 3 times the mean maximum exposure of M1 following the recommended 50 mg BID dosing, there were no clinically relevant effects on the QTc interval.

Clinical Pharmacokinetics

Tenapanor (parent drug) showed minimal systemic absorption with plasma concentrations below the lower limit of quantitation (0.5 ng/mL) in most PK samples in clinical trials. Consequently, it is not feasible to characterize the typical PK parameters of tenapanor following oral administration. Tenapanor is metabolized primarily by CYP3A. Plasma concentrations of the major metabolite (M1) were measured in clinical studies. There is minimal accumulation of M1 to steady-state as M1 C_{max} is approximately 13 ng/mL after a single dose of 50 mg tenapanor and 15 ng/mL at steady state following 50 mg BID in healthy subjects.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The dose-response results in the phase 2 study and the efficacy results in phase 3 trials overall support the acceptability of the proposed dosing regimen of tenapanor 50 mg BID. The results of the food effect study also support that tenapanor should be taken immediately before a meal, which is consistent with the design of the phase 3 clinical trials.

Therapeutic Individualization

Therapeutic individualization based on intrinsic or extrinsic factor is not necessary. Tenapanor has minimal systemic absorption following oral administration and the effectiveness of tenapanor to treat IBS-C is not dependent on its systemic exposure. Dose adjustment in patients with hepatic or renal impairment is not necessary. In vitro and in vivo drug interaction studies did not indicate clinically relevant drug-drug interaction potential for tenapanor.

Outstanding Issues

There are no outstanding issues that would preclude the approval of tenapanor from the Clinical Pharmacology perspective.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

Pharmacological properties and PK characteristics of tenapanor are summarized in Table 15 and Table 16.

Table 15. Summary of the General Pharmacology of Tenapanor

Pharmacology	
Mechanism of Action	Tenapanor is a locally acting inhibitor of NHE3. By inhibiting NHE3 on the apical surface of the enterocytes, tenapanor reduces absorption of sodium from the small intestine and colon, resulting in an increase in water secretion into the intestinal lumen, which accelerates intestinal transit time and results in a softer stool consistency.
Active Moieties	Tenapanor is the active moiety. The major metabolite (M1) is inactive against NHE3. No safety concerns related to M1 were found in animal toxicology studies. See Section 5 of this multidiscipline review for details of the nonclinical study results.
QT Prolongation	Based on extensive ECG data in healthy subjects (Study D5611C00005), no clinically relevant effects on the QTc interval was observed in doses up to tenapanor HCl 90 mg capsule BID (88 mg in tenapanor), which resulted in approximately 3 times the mean maximum exposure of M1 following tenapanor 50 mg BID in TEN-01-103, i.e., 48.7 ng/mL vs. approximately 15 ng/mL.

Abbreviation: BID, twice daily; ECG, electrocardiogram

Table 16. Summary of the Pharmacokinetics of Tenapanor

ADME	
Absorption	Tenapanor is minimally absorbed following oral administration. Plasma concentrations of tenapanor were below the lower limit of quantitation (0.5 ng/mL) in most samples (>99%) following single or repeated oral administration of tenapanor 50 mg in healthy subjects.
Distribution	Plasma protein binding Tenapanor: 99% M1: 97%
Metabolism	Tenapanor is metabolized primarily by CYP3A. Following oral administration of 15 mg radiolabeled ^{14}C-tenapanor in a mass balance study (D5611C00007) in healthy subjects, unchanged tenapanor was not detected in pooled plasma samples collected within 24 hours after dosing. M1 was the most abundant metabolite and accounted for 16% of plasma AUC_{0-24} in radioactivity.
Excretion	In the mass balance study (D5611C00007), the total radioactivity recovery was 89.2%, of which 79.3% was recovered in feces (65% in the unchanged drug) and 8.99% was recovered in urine as metabolites (1.5% as M1). The half-life of M1 is 18.7 hours.
General Information	
Bioanalysis	Validated LC-MS/MS methods were used to determine the concentrations of tenapanor and M1. See section 16.3.3 for details of the method validation.
Dose Proportionality	In healthy subjects (D5611C00005), C_{max} of M1 increased dose proportionally at tenapanor doses ranging from 14 mg to 88 mg formulated as a capsule.

Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; CYP, cytochrome P540; LC-MS/MS, liquid chromatography with tandem mass spectrometry

6.3.2. Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

Yes. The efficacy of tenapanor for the treatment of IBS-C was demonstrated in the two phase 3 trials. See Section 8 of this multidisciplinary review for details of study design and efficacy results of the phase 3 trials. The pharmacodynamic (PD) effect on fecal sodium excretion and dose-response relationships for efficacy described below provide supportive evidence of effectiveness. Note that tenapanor is a locally acting agent and the effectiveness of tenapanor to treat IBS-C is not dependent on its systemic exposure.

- *PD effect on fecal sodium excretion:* The PD results in the phase 1 study in healthy subjects (D5611C00005) showed that the mean daily fecal sodium excretion was greater in all tenapanor treatment groups compared to the placebo group (Table 17).

Table 17. Mean Daily Fecal Sodium Excretion by Tenapanor Doses After Repeated Dosing for 7 Days in Healthy Japanese Subjects (Study D5611C00005)

Cohort (Tenapanor Capsule Dose)	Placebo (n=14)	15 mg BID (n=12)	30 mg BID (n=12)	60 mg BID (n=12)	90 mg BID (n=12)
Mean ± SD (mEq/day)	4.1±3.1	21.3±17.8	32.2±16.8	28.4±23.4	30.8±15.6

The 15 mg, 30 mg, 60 mg, and 90 mg of tenapanor capsules correspond to 14 mg, 28 mg, 60 mg, and 88-mg dose of tenapanor, respectively.
Abbreviations: BID, twice daily; SD, standard deviation
Source of data: Table 24, Summary of Clinical Pharmacology

- *Dose-response for CSBM: once daily (QD) dosing regimen:* Tenapanor treatment improved weekly complete spontaneous bowel movement (CSBM) frequency compared to the placebo group in the phase 2a dose-finding study in patients with IBS-C (RDX5791-201). Following multiple doses of tenapanor 10, 30, or 100 mg QD and placebo for 4 weeks, the mean changes from baseline in weekly CSBM frequency were 1.50, 1.54, and 1.57 in the tenapanor 10 mg, 30 mg, and 100 mg QD dose groups, respectively, compared to 1.01 in the placebo group. Although there was no apparent dose-response relationship for change in weekly CSBM frequency among the tenapanor dose groups at Week 4, the 100 mg QD group overall showed the greatest effect in improving weekly CSBM frequency at time points prior to Week 4, suggesting a faster onset of treatment effect at higher doses. See OCP appendixes (Section 16.3) individual study report for details.
- *Dose-response for CSBM: twice daily (BID) dosing regimen:* A dose-response relationship was demonstrated in the phase 2b dose-finding study in patients with IBS-C (D5612C0001) in terms of overall CSBM responder rate (the primary endpoint) and change in weekly CSBM frequency (the secondary endpoint; Table 18). The tenapanor 50 mg BID dosing regimen showed greater treatment effect than the 5 mg BID and 20 mg BID dosing regimens which supported the dose selection for phase 3 trials.

Table 18. Dose-Response for CSBM in Study D5612C00001

Response	Placebo (n=89)	Tenapanor 5 mg BID (n=87)	Tenapanor 20 mg BID (n=87)	Tenapanor 50 mg BID (n=87)
CSBM response rate (%)	33.7	40.2	43.7	60.7*
Change in CSBM frequency (mean ± SD)	1.1±2.4	1.8±2.7	2.3±3.6	2.9±4.0

CSBM responders are defined as weekly responders with an increase of ≥1 CSBM from baseline for 6 out of 12 treatment weeks.
Change in CSBM frequency represents the change in average weekly CSBM from baseline to Week 12.
* Note that the treatment comparison of 50 mg BID group versus placebo was statistically significant (p<0.001).
Abbreviations: BID, twice daily; CSBM, complete spontaneous bowel movement; SD, standard deviation
Source of data: Tables 6 and 7, CSR D5612C0001

Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes. The efficacy and safety data from phase 3 trials overall support that the proposed tenapanor 50 mg BID dosing regimen is acceptable for the treatment of IBS-C. The dose selected for phase 3 trials are also supported by the dose-response results in phase 2 trials (RDX5791-201 and D5612C0001) as described above.

Dosing Timing Relative to a Meal

The proposed dosing regimen includes a recommendation to take tenapanor immediately prior to the first meal of the day and dinner, which is consistent with the study design of the phase 3 trials. The results of the food effect study in healthy subjects (D5611C00003, Part A) also showed that taking tenapanor immediately before a meal achieved greater PD effect on fecal sodium excretion compared to taking tenapanor after meals or in fasting conditions. In Study D5611C00003, the mean 24-hour fecal sodium excreted were 14.1 mEq, 17.2 mEq, and 25.9 mEq when tenapanor HCl 15 mg (14 mg in tenapanor) BID was administered for 4 days in a fasting condition, after meals, or 5 to 10 minutes prior to meals, respectively.

Dose-Response for Safety

Based on the integrated summary of safety with the integrated safety analysis set, the most commonly occurring treatment-emergent adverse events (TEAEs) in IBS-C subjects was diarrhea, occurring in 14.8% of IBS-C patients who received tenapanor 50 mg BID compared to 2.3% of patients administered placebo (see Section 10 for the review of safety). There were apparent dose-response relationships for diarrhea in phase 2 trials with higher incidence of diarrhea at higher doses.

- In the phase 2a study (RDX5791-201) for 4-week period, the incidence of diarrhea was 2.2% (N=46), 4.3% (N=47) and 4.3% (N=46) at tenapanor doses of 10 mg, 30 mg, and 100 mg QD, respectively, compared to an incidence of 2.1% (N=47) in the placebo group.
- In the phase 2b study (D5612C00001) for 12-week period, the incidence of diarrhea was 8.0% (N=88), 12.4% (N=89) and 11.2% (N=89) at tenapanor doses of 5 mg, 20 mg, and 50 mg BID, respectively, compared to an incidence of 0% (N=90) in the placebo group.

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

No. There is no need for alternative dosing regimen for subpopulations based on intrinsic factors. As discussed below, dose adjustment for renal or hepatic impairment is not necessary.

Renal Impairment

A dedicated renal impairment study for tenapanor was not conducted. Dose adjustment for renal impairment is unnecessary, given the following considerations:

- Tenapanor has minimal systemic absorption and only a small fraction of dose is excreted in urine (~9% of dose in metabolites).
- The Applicant provided safety data from patients with ESRD (Studies D5611C00001, D5613C00001, and TEN-02-201) and patients with chronic kidney disease (CKD) (Study D5610C00001) to support the use of tenapanor in renal impairment. PK data in renal impairment was available from Study D5611C00001, where ESRD patients on dialysis (eGFR <15 mL/min/1.73m^2) received tenapanor HCl (b) (4) The mean plasma concentrations of M1 in ESRD patients was 2.55 ng/ml (n=5), in comparison to 7.99 ng/mL (n=12) in healthy subjects at comparable doses.

Hepatic Impairment

A dedicated hepatic impairment study for tenapanor was not conducted. As tenapanor is minimally absorbed and primarily excreted in feces in unchanged form (~65% of dose), dose adjustment in hepatic impairment is not needed.

Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

No. The in vitro and in vivo DDI study results did not indicate clinically relevant DDI effect of tenapanor.

In Vivo Study Results

- Tenapanor is metabolized via CYP3A. In Study TEN-01-104, co-administration of tenapanor 50 mg with itraconazole (a strong CYP3A inhibitor) decreased systemic exposure to M1 by 50%. Plasma concentration of tenapanor was below LLOQ in most plasma samples either with or without itraconazole. As M1 is pharmacologically inactive and no safety concern was found in nonclinical studies, 50% decreased M1 exposure is not considered clinically significant.
- In TEN-01-103, co-administration of tenapanor 50 mg BID did not alter PK of midazolam (a CYP3A substrate) or cefadroxil (a PepT1 substrate).

In Vitro Drug-Drug Interactions Results

- Tenapanor is a substrate of CYP3A4 and CYP3A5; however, none of the CYP enzymes was found to be responsible for ≥25% of its overall elimination.
- Neither tenapanor nor M1 inhibits CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6. Tenapanor and M1 do not induce CYP1A2 and CYP2B6.
- Tenapanor is not a substrate of P-gp; however, M1 is a substrate of P-gp.
- Tenapanor and M1 are not substrates of BCRP, OATP1B1, or OATP1B3.
- Neither tenapanor nor M1 inhibits P-gp, BCRP, OATP1B1, and OATP1B3. M1 did not inhibit OAT1, OAT3, OCT2, MATE1, and MATE2-K.

7. Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

Table 19. Listing of Clinical Trials Relevant to this NDA/BLA

Trial Identity	Trial Design	Regimen/ Schedule/ Route	Study Endpoints	Treatment Duration/ Follow Up	No. of patients (Total *Tenapanor* *Placebo*)	Study Population	No. of Centers and Countries
	Controlled Studies to Support Efficacy and Safety						
TEN-01-301	Randomized 1:1, double-blind, placebo-controlled	50 mg/BID/Orally	6/12-week overall responder (weekly responder for at least 6/12 weeks for both CSBM and abdominal pain)	12 weeks/ 4 weeks	610* *309* *301*	Females and Males, 18 to 75 years of age	122 United States
TEN-01-302	Randomized 1:1, double-blind, placebo-controlled	50 mg/BID/Orally	6/12-week overall responder (weekly responder for at least 6/12 weeks for both CSBM and abdominal pain)	26 weeks	620* *293* *300*	Females and Males, 18 to 75 years of age	92 United States
D5612C0001	Randomized 1:1:1:1, double-blind, placebo-controlled	5 mg/BID/Orally 20 mg/BID/Orally 50 mg/BID/Orally	6/12-week overall responder (weekly responder for at least 6/12 weeks for both CSBM and abdominal pain)	12 weeks/ 4 weeks	356 *266* *90*	Females and Males, 18 to 75 years of age	79 United States
RDX5791-201	Randomized, 1:1:1:1, double-blind, placebo-controlled	10 mg RDX5791 30 mg RDX5791 100 mg RDX5791	Change in weekly complete spontaneous bowel movement (CSBM) frequency from the 14 day pretreatment baseline period to the end of the 4-week treatment period	4 weeks/ 2 weeks	186 *139* *47*	Females and Males, 18 to 75 years of age	43 United States
	Studies to Support Safety						
TEN-01-303	Open-label safety extension	50 mg/BID/Orally	Adverse events	39 weeks (after TEN-01-301) 26 weeks (after TEN-01-302)	312 *312* *0*	Females and Males, 18 to 75 years of age	122 United States

Trial Identity	Trial Design	Regimen/ Schedule/ Route	Study Endpoints	Treatment Duration/ Follow Up	No. of patients (Total *Tenapanor* *Placebo*)	Study Population	No. of Centers and Countries
Other studies pertinent to the review of efficacy or safety (e.g., clinical pharmacology studies)							
D5610-C00001	Randomized 1:1:1:1, double-blind, placebo-controlled	5 mg/BID/Orally 15 mg/BID/Orally 30 mg/BID/Orally 60 mg/BID/Orally Dose titration	Changes in urine albumin-to-creatinine ratio from baseline to Week 12	12 weeks/ 2 weeks	154 *77* *77*	Females and Males, 18 to 75 years of age	43 United States 5 Germany
D5611C00001	Randomized 1:1, double-blind, placebo-controlled	(b) (4)		4 weeks/ 2 weeks	88 *41* *32*	Females and Males, 18 to 75 years of age	14 United States
D5613C00001	Randomized 1:1:1:1:1:1, double-blind, placebo-controlled	1 mg/BID/Orally 3 mg/BID/Orally 10 mg/BID/Orally 30 mg/BID/Orally 3 mg/OD/Orally 30 mg/OD/Orally	Change in serum phosphate levels from end-of-washout to end-of-treatment/early termination in end-stage renal disease patients on hemodialysis	4 weeks/ 2 weeks	162 *135* *26*	Females and Males, 18 to 75 years of age	47 Poland Slovakia United Kingdom United States
TEN-02-201	Randomized 1:1 double-blind, 8-week, parallel group study with a 4-week, placebo-controlled, randomized withdrawal period	3 mg BID 10 mg BID Dose titration	Change in serum phosphorus from the end of the 8-week treatment period to the end of the 4-week randomized withdrawal period	8 weeks/ 4 weeks	300 *218* *82*	Females and Males, 18 to 75 years of age	41 United States

*629 patients were randomized to study 301, and 620 to study 302. Due to GCP violations at site 284, patients from that site were excluded from analyses. For details refer to section on "Issues regarding data integrity and submission quality" below (7.3).
Abbreviations: BID, twice daily; CSBM, complete spontaneous bowel movement; OD, every other day
Source: Reviewer's table, electronic common technical document: \\CDSESUB1\evsprod\NDA211801\211801.enx

7.2. Review Approach

Efficacy evaluation focused on the two randomized, double-blind, placebo-controlled confirmatory studies (Study TEN-01-301 and Study TEN-01-302), which were independently assessed. No pooling of results for efficacy analysis was conducted.

7.3. Data Integrity

Compliance with Good Clinical Practice

The applicant attested that studies were conducted in compliance with Good Clinical Practice.

Financial Disclosures

Form 3454 was reviewed and indicated no reportable financial conflicts of interest between the applicant and any investigator involved in the conduct of the studies contained in the application.

Issues Regarding Data Integrity and Submission Quality

The submission quality of this application was adequate in organization, ease of finding information and completeness of information. The Applicant responded appropriately to requests for information. Prior to submission of the application, the Applicant discovered serious breaches in GCP at one of the sites, site 284. Identified issues included lack of adequate oversight by the Principal Investigator (PI), questionable signatures attributed to this PI, inadequate site resources and personnel to carry out the responsibilities of conducting the trial procedures. The FDA visited the site and the Applicant conducted an audit. Based on the findings of the audit, data from this site were excluded from further analyses, including all safety and efficacy analyses.

The review team identified two additional sites (sites 202 and 180) that had outlier safety results. Site 202 had no reported AEs but only enrolled seven patients, four in one arm and three in the other. Because of the small numbers of patients, it is possible that AEs did not occur. The other site, Site 180, reported very high efficacy (68% for overall responder rate in Study 302, 24% higher than placebo) and very low rates of AEs (one patient with an AE of common cold in 19 patients). This site was inspected by the Office of Scientific Investigations. Refer to Section 4.1 above. The preliminary classification was Voluntary Action Indicated for protocol violations (failure to dispense drug at 2 visits for 2 patients, failure to record abnormal laboratory findings as adverse events, failure to provide a subject screening log to the ORA investigator during the inspection). Deviations identified were considered minor and were not anticipated to impact the interpretability of data from the site.

8. Review of Relevant Individual Trials Used to Support Efficacy and Safety

8.1. Study Design of Key Studies That Support Approval

8.1.1. Phase 3 Studies: TEN-01-301 (Study 301) and TEN-01-302 (Study 302)

Description of Both Studies

(**Note:** Descriptions in this section are extracted from the Applicant's clinical study report. In addition, from this point on in this review, we will refer to Study TEN-01-301 as Study 301 and Study TEN-01-302 as Study 302.)

Both studies had similar study objectives, primary and secondary endpoints, primary analysis populations, statistical analysis plan and sample size calculations. The differences between the two studies were that Study 301 had a 12-week double -blind treatment period with a 4-week randomized withdrawal period, whereas Study 302 had a 26-week double-blind treatment period. In both studies, the primary endpoint was assessed based on 12 weeks of treatment. The follow up duration for Study 301 was 4 weeks, but Study 302 included up to an additional 14 weeks of follow up. Both studies were conducted in the United States.

Study Objective

The primary objective of the studies was to assess the efficacy of tenapanor 50 mg for the treatment of IBS-C when administered BID for the duration of each study.

The secondary objective was to assess the safety and tolerability of tenapanor 50 mg when administered BID for the duration of each study.

Study Design

Both studies were phase 3, randomized, double-blind, placebo-controlled studies of tenapanor in adults (18 to 75 years old) with IBS-C (as defined by the Rome III Criteria for the Diagnosis of IBS). Study 301 was conducted in 122 sites and Study 302 in 92 sites in the United States.

Study 301 consisted of a 2-week screening period, a 12-week treatment period, and a 4-week randomized-withdrawal (RW) period. Approximately 2 weeks prior to study randomization, prospective subjects were assessed for the eligibility of the study. Those that met the basic requirements were asked to provide a written informed consent. Subjects were required to discontinue the use of all prohibited medications[1] at Visit 1 (Screening/Day -14) for the duration of the study. During the screening period, subjects self-reported information about the status of their IBS symptoms daily via a touch-tone telephone diary (interactive voice response system

[1] Prohibited medications included laxatives and stool softeners (unless stable during screening period and continued throughout trial), other treatments for IBS, and various other medications anticipated to have an effect on stool frequency or consistency (including narcotics, anticonvulsants, antidepressants, antipsychotics, anticholinergics, etc).

[IVRS] diary). This included information about their stool frequency, stool consistency, straining, abdominal pain, abdominal discomfort, abdominal bloating, abdominal fullness, abdominal cramping, and rescue medication[2] usage. IBS severity and constipation severity were collected weekly through the IVRS diary.

Randomization occurred twice with a ratio of 1:1 (tenapanor: placebo); at Visit 2 for the 12-week treatment phase and at Visit 6 for the RW period (Study 301 only). A computer-generated randomization schema was centrally available via an interactive web response system (IWRS) to all clinical sites that met the requirements for participation in the study.

During the 12-week double-blind treatment period and the double-blind RW period, subjects continued to record daily and weekly assessments via the IVRS diary. Scheduled visits took place at Weeks 2, 4, 8, and 12 (Days 15, 29, 57, and 85) during the treatment period and Week 2 (Day 99) of the RW period for study assessment visits (See Table 85 in the Appendix). Subject compliance with study drug was monitored closely by clinical site staff throughout the study. Subjects returned to the site at the end of the 4-week RW period (Week 16, Day 113) to obtain safety and other end of study information.

Study 302 consisted of a 2-week screening period, and a 26-week treatment period. The procedures from screening (Day -14) to randomization (Visit 2, Day 0) were identical to those described for Study 301 above.

Subjects were randomized with a ratio of 1:1 (tenapanor: placebo) according to a centrally available computer-generated randomization schema. During the 26-week double-blind treatment period, subjects continued to record daily and weekly assessments via the IVRS diary as instructed. Scheduled visits took place at Weeks 2, 4, 8, 12, 16, 20, and 26 (Days 15, 29, 57, 85, 113, 141, and 183) during the treatment period (See Table 86 in the Appendix). Subject compliance with daily diary entries was monitored on an ongoing basis by the clinical site staff. Subject compliance with study drug was also monitored closely by clinical site staff throughout the study.

Primary and Secondary Efficacy Endpoints (Studies 301 and 302)

The primary efficacy endpoint of both studies was the proportion of subjects who were weekly overall responders at 6 or more of the first 12 weeks of double-blind treatment (6/12 weeks overall responder rate).

An overall responder was defined as a weekly responder for at least 6/12 weeks where both complete spontaneous bowel movement (CSBM) and abdominal pain response criteria were met for the week. The CSBM and abdominal pain response criteria are defined below.

The CSBM response criterion was defined as an increase of one or more in average weekly CSBMs from baseline. A CSBM was defined as an SBM for which the subject responded “yes” to the following question; “Did you feel like you completely emptied your bowels?” Any SBM which was preceded within 24 hours by the use of rescue medication was not counted as an

[2] Rescue medication use was limited to Bisacodyl (5mg tablet or 10mg suppository) for protocol specified symptoms of severe constipation defined as at least 72 hrs from last BM, or intolerable symptoms.

SBM, and therefore, also not counted as a CSBM as defined above. Should a subject not have data reported for a given week (either due to a gap in reporting or due to discontinuation), the subject was considered to be a nonresponder for the week. The average weekly CSBMs was calculated as the sum of the number of CSBMs reported during each day of the defined weekly period divided by the number of days CSBMs were reported multiplied by 7. A valid week required at least 4 days of SBM reporting.

The abdominal pain response criterion was defined as a decrease of 30% or more in average weekly worst abdominal pain from baseline. Patients were asked to report daily on their "worst abdominal pain over the past 24 hours." Abdominal pain was scored using the scale 0 (no pain) to 10 (very severe pain). The average weekly abdominal pain score was calculated as the average score for all days during a valid week. A valid week required at least 4 days of abdominal pain reporting. Should a subject not have data reported for a given week (either due to a gap in reporting or due to discontinuation), the subject was considered to be a nonresponder for the week.

The Applicant pre-specified a total of five multiplicity-controlled secondary endpoints in study 301 listed below:

- 6/12 weeks overall CSBM responder.
- 6/12 weeks overall abdominal pain responder.
- 9/12 weeks overall responder.
- 9/12 weeks overall CSBM responder
- 9/12 weeks overall abdominal pain responder

The Applicant pre-specified a total of eight multiplicity-controlled secondary endpoints in study 302. These included:

- 6/12 weeks overall CSBM responder.
- 6/12 weeks overall abdominal pain responder.
- 9/12 weeks overall responder
- 9/12 weeks overall CSBM responder
- 9/12 weeks overall abdominal pain responder
- 13/26 weeks overall responder rate
- 13/26 weeks overall CSBM responder rate
- 13/26 weeks overall abdominal pain responder rate

The 9/12 week response criterion was defined as meeting the same responder criteria as those for the 6/12 week responder for 9 out of the 12 weeks. Additionally, for the 9/12 week response criteria, the average weekly CSBMs had to be ≥3.

In addition, although not included in the multiple testing procedure, the applicant defined an endpoint of "durable overall response," which the review team also evaluated. "Durable overall response" was defined as a patient who met criteria for 9/12 week response, where additionally, 3 of the last 4 weeks had to be responder weeks. FDA considers this endpoint clinically relevant because it assesses the durability of response (albeit limited to 12 weeks) to

therapy and ensures tachyphylaxis did not occur. This endpoint demonstrates that the improvement seen in the 12 week period was not limited only to the first 6 weeks and then no further efficacy thereafter. With this single exception, this review otherwise will focus only on the primary and key secondary endpoints.

Clinical Outcome Assessments

Patient reported outcome measures were utilized in assessing the primary endpoint, derived from electronic diary data as outlined above. The questions used to assess abdominal pain and stool frequency were considered fit-for-purpose. Additional concepts assessed by the applicant included straining, abdominal bloating, discomfort, fullness, cramping, and stool consistency. Validity of assessing the concepts of bloating, discomfort, fullness, and cramping were questioned by the review team, given potential overlap between these symptoms with abdominal pain measured in the primary endpoint. (b) (4) The review team requested input from COA staff in interpreting results of the change from baseline in straining score, given that straining has been suggested as a clinically relevant concept. The anchor based analysis concluded that no meaningful difference in straining was demonstrated in patients treated with tenapanor. Refer to full COA consult review by Susan Pretko for additional detail.

Statistical Analysis Plan:

Determination of Sample Size for Both Studies

The Applicant expected that a sample size of 300 in each treatment group would achieve 95% power to detect a difference of 0.15 (15%) between tenapanor 50 mg BID and placebo in 6/12 week overall responder rates (assuming that tenapanor 50 mg BID response rate was at least 45% under the alternative hypothesis, and the response rate in the placebo group was no closer than 15% from tenapanor 50 mg BID). The test statistic used for sample size estimation was the two-sided Fisher's exact test with significance level of 0.05. This sample size also had 80% power to detect an 11.6% difference in responder rates between the treatment groups when the responder rates were in the same range as above.

Analysis Populations

The Intent-to-Treat (ITT) analysis set consisted of all subjects who met the study entry inclusion/exclusion criteria, received at least one dose of study drug, and had at least 1 valid week of efficacy assessment data (minimum of 4 days). Although the Applicant referred to this as the ITT set, this is actually a modified ITT population given that it excludes patients based on post-randomization variables that may be affected by treatment assignment (i.e., whether the patient had at least one valid week of efficacy assessment data).

The Per-Protocol (PP) analysis set consisted of all subjects included in the ITT analysis set who completed the study as planned with no major protocol deviations.

The Applicant used the ITT analysis set for the analysis of the primary and key secondary efficacy variables. Subjects were analyzed according to the treatment group into which they were randomized.

Handling of Missing and Imputing the Data

For the primary analysis, patients with a week with less than 4 diary days were treated as a nonresponder for that week. If there were at least 4 nonmissing diary days in a given week, weekly SBMs and CSBMs were calculated based on the available diary days so that missing days during the week were imputed with the mean for the nonmissing days.

In addition, upon request by FDA, the applicant conducted a sensitivity analysis with alternate handling of missing data. Specifically, instead of imputing a mean score for CSMB on days with missing data, days with missing diary data for bowel movements were imputed with "zero," and weeks with <4 of 7 days of data were considered nonresponder weeks. Analyses of the primary endpoint utilizing this alternate imputation method were considered as sensitivity analysis.

Methods of Pooling Sites into Groups

For the purpose of adjusting for investigator effects in statistical models, the Applicant pooled investigator sites into groups based on geographic region and number of subjects enrolled with an aim for comparable sample sizes amongst pooled investigator sites. As indicated in the study protocol, the goal of the pooling strategy was to avoid less than a minimum number of subjects per pooled investigator site. The size of a pooled investigator site was to be generally no larger than the total number of subjects enrolled at the highest enrolling individual investigator site. The pooled investigator sites were used in all applicable analyses where adjustment for investigator effect was desired.

Based on an average of six subjects per site to be enrolled, the primary pooled investigator site strategy targeted 10 pools of approximately 60 subjects each (approximately 30 per treatment group per pooled investigator site). As a sensitivity analysis, a second pooling had a target of 20 pools of approximately 30 subjects each (approximately 15 per treatment group per pooled investigator site). The actual designation of membership in a pooled investigator site was not made until the final enrollment quantities and final number of sites used was completed. The final pooling strategy was defined before treatment unblinding.

Statistical Methods

Statistical analyses were performed at the two-sided significance level of 0.05. For analyses of all efficacy variables involving responder rates or proportions, the Applicant used a Cochran-Mantel-Haenszel (CMH) test with pooled investigator site as a stratification adjustment variable. Summary statistics included the pairwise risk difference for tenapanor 50 mg BID compared with placebo along with the asymptotic 95% confidence interval (CI). The adjusted relative risk (adjusted for pooled investigator site) was based on the ratio of responder rates for tenapanor 50 mg BID versus placebo. The 95% CI versus placebo was presented for the adjusted relative risk.

Multiple Testing Procedure

For both studies (301 and 302), a sequential testing procedure was utilized to control the family-wise type 1 error rate associated with the primary efficacy variable and key secondary efficacy variables. The primary efficacy variable was tested at the 2-sided 5% level of significance. If this test was significant, the first key secondary efficacy variable was tested at the 5% level. If this test was significant, then the next key secondary efficacy variable was tested at the 5% level. This procedure continued until one of the key secondary variables in the list resulted in a p-value of >5%. Key secondary efficacy variables up to that point in the list were declared statistically significant.

8.1.2. Safety Extension: TEN-01-303 (Study 303)

Study 303 was an open-label, long term extension study that was included to ensure an adequate number of patients had long term (52 week) exposure to tenapanor to inform the safety of chronic administration. No efficacy evaluation was conducted during this trial. The study design is briefly reviewed here, as these patients are analyzed in the integrated safety analysis described in the sections that follow.

The study was an open-label, long-term extension study which enrolled 312 patients. To be eligible for inclusion, patients must have either 1) completed all 16 weeks of Study 301, OR 2) completed all 26 weeks of Study 302 (as described above in Section 8.1.1). All patients who enrolled received 50 mg twice daily of tenapanor, as open-label treatment. The primary objective of the study was to assess safety of tenapanor when given chronically for up to 52 weeks.

Site visits occurred every 13 weeks (which included measurement of vital signs, review of concomitant medications, pregnancy test when indicated, and solicitation of adverse events), and telephone follow-up occurred at intervening timepoints (which included review of AEs and concomitant medications), such that patients were in contact with the site approximately every 4 weeks. Physical examination and an additional ECG were conducted at final visit (or early termination if applicable). Duration of treatment was 26 weeks for subjects entering from Study 302 (to provide a total of 52 weeks cumulative exposure) and approximately 39 weeks (±2) for patients entering from Study 301. Appropriate safety assessments were collected throughout the study (for details refer to Applicant's clinical study report for study 303).

9. Review of Efficacy

In this section, efficacy results of each phase 3 study are reported separately.

9.1.1. TEN-01-301 (Study 301) Results

Patient Disposition

A total of 1,599 subjects were screened and 629 were randomized into the 12-week treatment period. Overall, 96 (15.3%) subjects withdrew prior to completing the 12-week treatment period, with the most common reasons for withdrawal being withdrawal of consent (34 [5.4%] subjects), AEs (26 [4.1%] subjects), and loss to follow-up (18 [2.9%] subjects). The most common AE leading to discontinuation was diarrhea. The proportion of subjects who dropped out of the study was higher on tenapanor (18%) than placebo (12%), primarily due to a greater proportion who discontinued due to AE (7.5% versus 0.6%).

Table 20 shows the disposition of the subjects for Study 301.

Table 20. Subject Disposition, 12-Week Treatment Period (All Subjects), Study 301

Category	Placebo	Tenapanor 50 mg BID	Overall
Screened – n			1599
Screen failure – n (%)[1]			970 (60.7)
Primary reason for screen failure – n (%)[1]			
Did not meet protocol Inclusion/Exclusion criteria			181 (11.3)
Did not meet PRO/diary			751 (47.0)
Withdrawal of consent			26 (1.6)
Lost to follow-up			5 (0.3)
Other			7 (0.4)
Randomized – n (%)[1]	310 (19.4)	319 (19.9)	629 (39.3)
Completed the 12-week treatment period – n (%)[2]	272 (87.7)	261 (81.8)	533 (84.7)
Withdrew prior to completing the 12-week treatment period – n (%)[2]	38 (12.3)	58 (18.2)	96 (15.3)
Primary reason for early withdrawal – n (%)[2]			
Adverse event	2 (0.6)	24 (7.5)	26 (4.1)
Unsatisfactory response to study treatment	1 (0.3)	2 (0.6)	3 (0.5)
Non-compliance with study treatment	4 (1.3)	2 (0.6)	6 (1.0)
Decision by the Investigator or Sponsor	4 (1.3)	3 (0.9)	7 (1.1)
Consent withdrawn	14 (4.5)	20 (6.3)	34 (5.4)
Protocol violation	1 (0.3)	0	1 (0.2)
Lost to follow-up	11 (3.5)	7 (2.2)	18 (2.9)
Death	0	0	0
Other	1 (0.3)	0	1 (0.2)
Safety Analysis Set – n (%)[2]	301 (97.1)	309 (96.9)	610 (97.0)
Reasons excluded from Safety Analysis Set – n (%)[2]			
Did not receive any study drug during 12-week treatment period	1 (0.3)	0	1 (0.2)
Serious breach of GCP	8 (2.6)	10 (3.1)	18 (2.9)
ITT Analysis Set – n (%)[2]	299 (96.5)	307 (96.2)	606 (96.3)
Reasons excluded from ITT Analysis Set – n (%)[2]			
Did not meet Inclusion/Exclusion criteria	2 (0.6)	2 (0.6)	4 (0.6)
Did not receive any study drug during 12-week treatment period	1 (0.3)	0	1 (0.2)
Serious breach of GCP	8 (2.6)	10 (3.1)	18 (2.9)
PP Analysis Set – n (%)[2]	242 (78.1)	225 (70.5)	467 (74.2)
Primary reason for PP Analysis Set exclusion – n (%)[2]			
Excluded from ITT Analysis Set during 12-week treatment period	11 (3.5)	12 (3.8)	23 (3.7)
Major protocol violations	8 (2.6)	8 (2.5)	16 (2.5)
Inadequate daily IVRS diary assessments	45 (14.5)	63 (19.7)	108 (17.2)
Study drug compliance	4 (1.3)	11 (3.4)	15 (2.4)

Abbreviations: BID = Twice daily; GCP = Good Clinical Practice; ITT = Intent-to-treat; IVRS = Interactive voice response system; N = Number of subjects randomized; n = Number of subjects analyzed; PP = Per-protocol; PRO = Patient reported outcomes.

1 Percentages were calculated using the number of subjects screened as the denominator.

2 Percentages were calculated using the number of subjects randomized as the denominator.

Source: Applicant's Clinical Study Report; Page58/1530

Demographics and Baseline Characteristics

Table 21 below summarizes demographic and baseline characteristics of patients enrolled in Study 301. The study population was generally representative of the IBS-C population, including that it enrolled a greater proportion of females (81%) than males, consistent with the distribution of IBS. Racial distribution was reasonable, including 31% African Americans, an often under-represented group. Mean age was 45 years and the range spanned from 18 to 75 years of age, with adequate geriatric representation. Demographic and baseline characteristics were generally similar between treatment groups.

Table 21. Summary of Demographic and Baseline Characteristics, 12-Week Treatment Period (ITT Analysis Set), Study 301

Characteristic	Placebo (N = 299)	Tenapanor 50 mg BID (N = 307)	Overall (N = 606)
Age at informed consent (years)			
n	299	307	606
Mean (SD)	44.9 (13.04)	45.0 (13.38)	45.0 (13.20)
Median	45.0	45.0	45.0
Minimum – Maximum	18 - 75	18 - 73	18 – 75
Gender – n (%)			
Male	50 (16.7)	63 (20.5)	113 (18.6)
Female	249 (83.3)	244 (79.5)	493 (81.4)
Race – n (%)			
White	186 (62.2)	201 (65.5)	387 (63.9)
Black or African American	100 (33.4)	88 (28.7)	188 (31.0)
Asian	4 (1.3)	10 (3.3)	14 (2.3)
American Indian or Alaskan Native	2 (0.7)	0	2 (0.3)
Native Hawaiian or Other Pacific Islander	0	0	0
Multiple[1]	5 (1.7)	6 (2.0)	11 (1.8)
Unknown	0	0	0
Other	2 (0.7)	2 (0.7)	4 (0.7)
Ethnicity – n (%)			
Hispanic or Latino	83 (27.8)	98 (31.9)	181 (29.9)
Not Hispanic or Latino	216 (72.2)	209 (68.1)	425 (70.1)
Baseline weight (kg)			
n	299	307	606
Mean (SD)	80.10 (19.440)	81.89 (20.913)	81.01 (20.203)
Median	77.20	80.00	78.55
Minimum – Maximum	43.0 – 144.6	37.0 – 157.0	37.0 – 157.0
Baseline height (cm)			
n	299	307	606
Mean (SD)	165.21 (9.815)	165.53 (9.792)	165.37 (9.796)
Median	165.00	165.00	165.00
Minimum – Maximum	131.0 – 201.1	131.0 – 199.5	131.0 – 201.1
Baseline BMI (kg/m^2)			
n	299	307	606
Mean (SD)	29.30 (6.442)	29.88 (7.198)	29.60 (6.836)
Median	28.70	28.70	28.70
Minimum – Maximum	15.5 – 55.0	13.1 – 57.4	13.1 – 57.4

Abbreviations: BID = Twice daily; BMI = Body mass index; ITT = Intent-to-treat; N = Number of subjects randomized; n = Number of subjects analyzed; SD = Standard deviation.

Note: Baseline for body weight and BMI was defined as the measurement taken at Day 1 pre-dose. If missing, the last measurement prior to the first dose of study drug was used.

1 Subjects reported more than one race.

Source: Applicant's Clinical Study Report for Study 301; Page 63/1530

Efficacy Results: Primary Endpoint

A total of 192 (62.1%) subjects in the tenapanor 50 mg BID group and 200 (66.4%) subjects in the placebo group were compliant between 80% to 100% of times for study drug administration.[3] Compliance was generally similar between treatment groups.

In the 12-week treatment period, for the ITT population, 83 (27.0%) subjects in the tenapanor 50 mg BID group and 56 (18.7%) subjects in the placebo group were considered to be 6/12 week overall responders. The difference (95% CI) in 6/12 week overall responder rates for tenapanor versus placebo was 8.31% (1.66%, 14.96%), which was statistically significant (p=0.020) based on a Cochran-Mantel-Haenszel (CMH) test stratified by pooled investigator site.

The table below shows the primary efficacy results in the ITT population, and supportive analysis based on the PP population.

Table 22. Study 301 Primary Efficacy Endpoint Results, ITT Population and PP Population

6/12 weeks overall responder rate	Tenapanor	Placebo	P-Value	Difference (95% CI)
ITT (primary analysis)	83/307 (27.0%)	56/299 (18.7%)	0.020	8.3% (1.7%, 15.0%)
PP (exploratory analysis)	76/225 (33.8%)	54/242 (22.3%)	0.006	11.5% (3.4%, 19.6%)

Abbreviations: CI, confidence interval; ITT, intent-to-treat; PP, per-protocol
Source: TEN-01-301 Clinical Study Report Table 11–11 (pg. 79), Table 14.2.1.2 (pg. 469)

The Applicant's results were confirmed by the FDA.

As described above, where data were missing for a given day (within a week that had diary entries for at least 4/7 days) the missing days were imputed with the mean of the non-missing days per SAP. As an additional sensitivity analysis ("zero imputation method"), FDA requested analysis of the primary endpoint and key secondary endpoints, where days with missing data were imputed with zero for the CSBM that day. Utilizing this method, 27% of tenapanor treated patients were responders, vs 19% on placebo, consistent with the results in the ITT population (results in Applicant's response to IR received 4/23/19, TEN-01-301-Table 14.2.1.1s).

[3] Compliance with study drug was calculated as the total number of tablets dispensed, minus the total number of tablets returned, divided by 2 times the number of days during the treatment period, multiplied by 100.

Secondary Efficacy Analysis

Multiplicity-controlled secondary efficacy variables included:

- 6/12 weeks overall CSBM responder;
- 6/12 weeks overall abdominal pain responder;
- 9/12 weeks overall responder;
- 9/12 week overall CSBM responder rate;
- 9/12 week overall abdominal pain responder rate

"Durable overall response", although exploratory, was considered clinically relevant to be included in the review because it captures a clinically relevant concept (see Section 8.1, "Primary and Secondary Endpoints").

Table 23 presents the efficacy results for these endpoints using the same statistical analysis method as was utilized for the primary endpoint.

Table 23. Study 301 Secondary Endpoints Results, ITT Population

Secondary Endpoints in Order	Tenapanor	Placebo	P-Value[#]	Difference (95% CI)
Overall CSBM response (6/12)	104/307 (33.9%)	88/299 (29.4%)	0.270	4.4% (-3.0%, 11.8%)
Overall abdominal pain response (6/12)	135/307 (44.0%)	99/299 (33.1%)	0.008	10.9% (3.2%, 18.6%)
Overall response (9/12)	42/307 (13.7%)	10/299 (3.3%)	<0.001	10.3% (6.0%, 14.7%)
Overall CSBM response (9/12)	52/307 (16.9%)	15/99 (5.0%)	<0.001	11.9% (7.1%, 16.9%)
Overall abdominal pain response (9/12)	93/307 (30.3%)	58/299 (19.4%)	0.003	10.9% (4.1%, 17.7%)
"Durable overall response"	40/307 (13.0%)	10/299 (3.3%)	<0.001	9.7% 5.4%, 14.0%)

Abbreviations: CI, confidence interval; CSBM, complete spontaneous bowel movement; ITT, intent-to-treat
[#]Reported p-values are not adjusted for multiplicity
Source: TEN-01-301 Clinical Study Report Table 11-12 (pg. 81), Table 11-13 (pg.83), Table 11-14 (pg. 85), Table 11-15 (pg.87), Table 11-16 (pg. 88)

As shown in Table 23, the difference in the responder rates for the first secondary endpoint (overall CSBM response (6/12) between tenapanor and placebo groups was not statistically significant (p=0.27). Therefore, based on the pre-specified testing hierarchy for the secondary endpoints, the testing will stop and should not proceed to the analysis of the next endpoint. Consequently, the rest of the analyses for the multiplicity-controlled secondary endpoints are considered exploratory. These results were confirmed by the statistical reviewer.

Although exploratory, a descriptive analysis showing that a greater proportion of patients on tenapanor than placebo achieved what the applicant referred to as (b) (4)

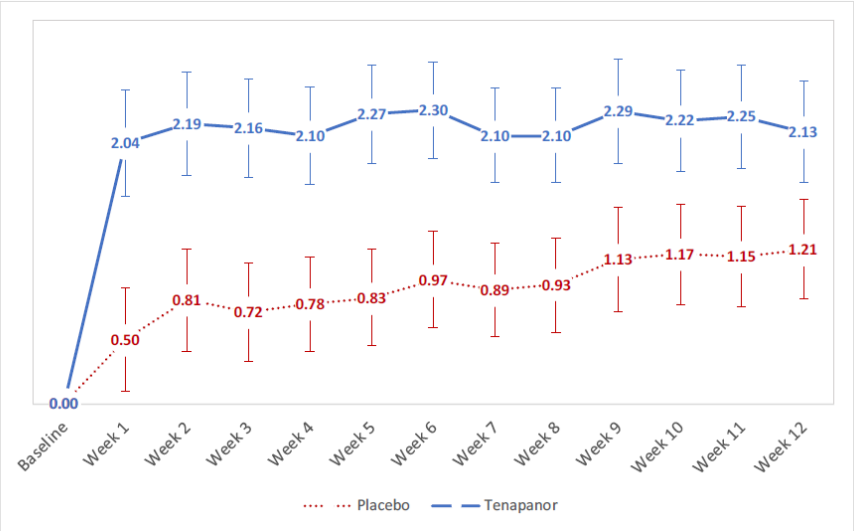
(b) (4)

As described above, sensitivity analyses (to missing data) of these endpoints using a "zero imputation" method, as requested by the Agency, showed results comparable to those demonstrated by the prespecified primary statistical method (data not shown, refer to Applicant's response to IR dated 4/23/19).

Time to Onset of Clinical Benefit

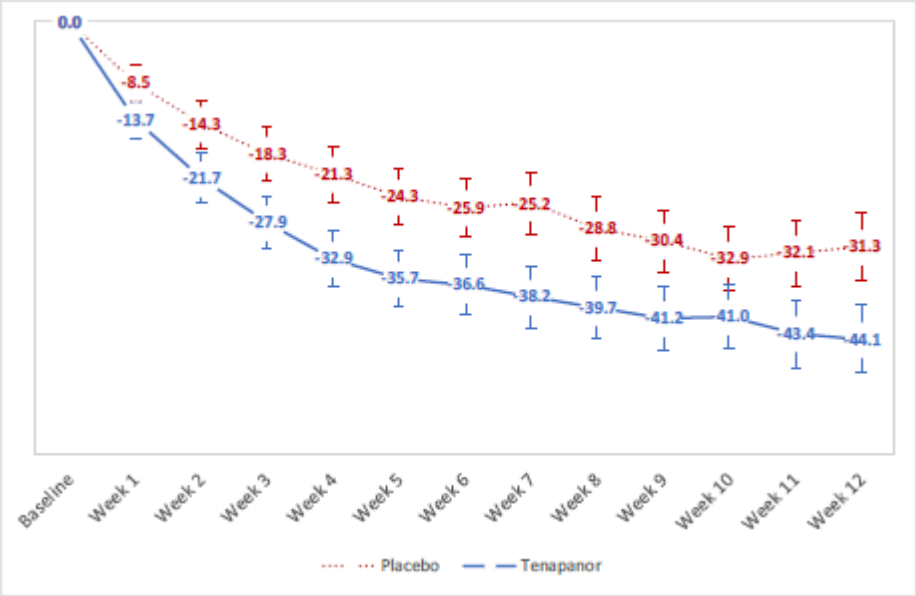
The onset of action of tenapanor was observed early in the 12-week treatment period with tenapanor arm demonstrating improvement in average change in both CSBM frequency as well as abdominal pain intensity by Week 1. Refer to Figure 2 and Figure 3 below.

Figure 2. Least Squares Mean and 95% CI for Change from Baseline in Average Weekly CSBMs (Study 301)- 12 week treatment period



Source: sponsor's response to IR received 8/7/19, ITT population from study 301, refer to CSR Table 14.2.3.1a
Remark: y-axis represents "Average Weekly CSBM"

Figure 3. Least Squares Means and 95% CI for Percent Change from Baseline in Average Weekly Abdominal Pain (Study 301) 12 week treatment period

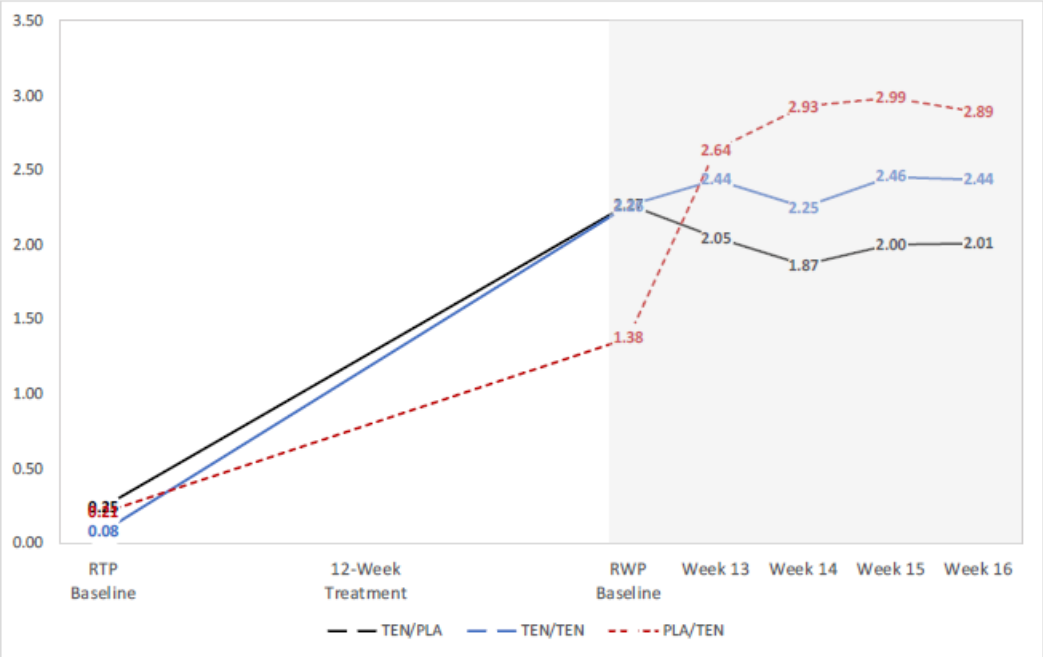


Source: sponsor's response to IR received 8/7/19, ITT population from study 301, refer to CSR Table 14.2.6.1a
Remark: y-axis represents "Average Weekly Abdominal Pain"

(b) (4)

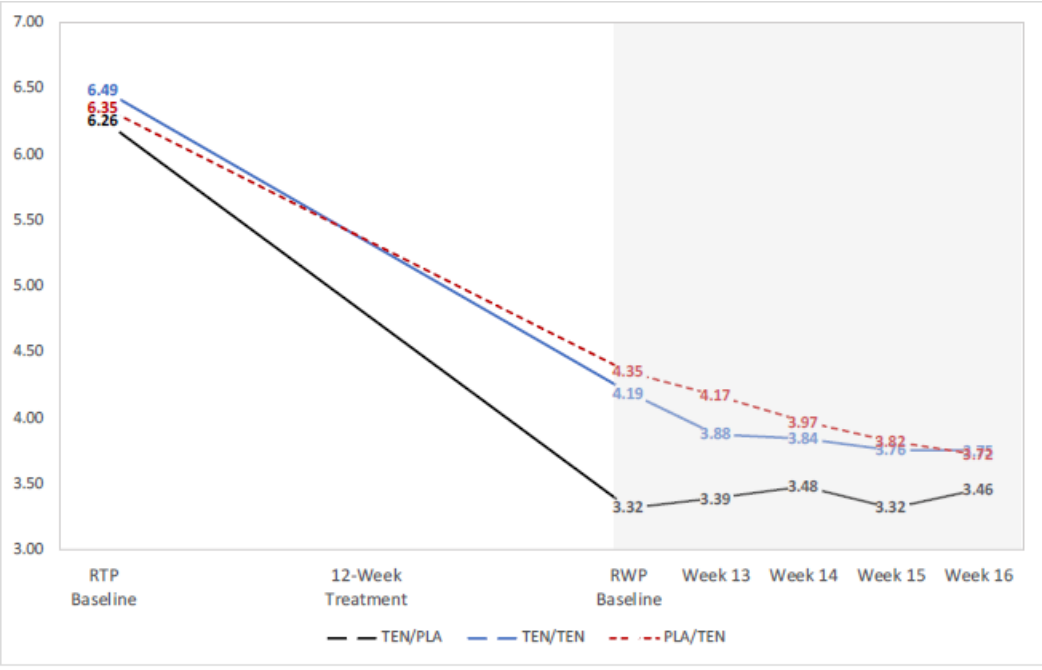
For the randomized withdrawal period, in patients re-randomized to placebo, CSBM frequency and abdominal pain severity worsened over the 4-week period on average but remained improved compared to baseline (refer to Figure 4 and Figure 5). Patients who continued on tenapanor maintained their response to therapy over the additional 4 weeks on average. Patients on placebo who were re-randomized to tenapanor had an average increase in CSBM frequency and a decrease in abdominal pain.

Figure 4. Study 301: Mean Weekly CSBM for Baseline of Randomized Treatment Period (RTP) and 4-Week Randomized Withdrawal Period (RWP)



Source: sponsor's response to IR received 8/7/19, ITT population from study 301, refer to CSR Table 14.2.3.1b
Remark: y axis represents mean average weekly CSBM rate

Figure 5. Study 301, Mean Weekly Abdominal Pain Score for Baseline of Randomized Treatment Period (RTP) and 4-Week Randomized Withdrawal Period (RWP)



Source: sponsor's response to IR received 8/7/19, ITT population from study 301, refer to CSR Table 14.2.6.1b
Remark: y axis represents mean weekly abdominal pain score

Subgroup Analyses

Table 24 below summarizes overall response rates (primary endpoint) by treatment arm and treatment differences with 95% CIs for gender, age category (<65 and ≥65) and race subgroups.

Table 24. Study 301 Subgroup Analyses by Gender, Age Category and Race for Primary Endpoint*

Subgroup	Tenapanor	Placebo	Difference (95% CI)
Gender			
Female	88/244 (36.1%)	79/249 (31.7%)	4.3% (-4.0%, 12.7%)
Male	16/63 (25.4%)	9/50 (18.0%)	7.4% (-7.7%, 22.5%)
Age Category			
<65	71/279 (25.5%)	52/277 (18.8%)	6.7% (- 0.2%, 13.6%)
≥65	12/28 (42.9%)	4/22 (18.2%)	24.7% (0.3%, 49.1%)
Race			
White	55/201 (27.4%)	28/186 (15.1%)	12.3% (4.3%, 20.3%)
Black	26/88 (29.6%)	24/100 (24.0%)	5.6% (-7.1%, 18.2%)
Asian	0/10 (0%)	1/4 (25%)	-25% (-67.4%, 17.4%)

* The primary endpoint was the 6/12 week overall responder rate (CSBM and abdominal pain combined)
Abbreviations: CI, confidence interval
Source: FDA analysis

In all subgroups, except the Asian race subgroup, response rates showed a trend in favor of tenapanor, with differences varying from 4.3% in the female subgroup to 24.7% in the elderly subgroup (>65 years), although for most of the subgroups, there was no significant difference between tenapanor and placebo (95% CI included zero). In the Asian subgroup, the treatment difference was in favor of placebo. However, the Asian subgroup had only 14 patients in total and only one patient out of 14 patients was a responder (placebo arm). In addition, a numerically greater estimated treatment effect was observed in the geriatric subgroup as compared to those <65 years. A similar trend was not observed in Study 302, suggesting that the Study 301 difference may be due to chance. In both cases (Asian race and age subgroup analyses), the numbers of patients in these subgroups are small, precluding definitive conclusions.

9.1.2. TEN-01-302 (Study 302) Results

Patient Disposition

A total of 1461 subjects were screened. The most common reason for screen failure was failure to meet the screening eligibility criteria for active IBS-C symptoms (665 [45.5%] subjects).

Overall, 620 (42.4%) subjects were randomized (306 [20.9%] subjects into the tenapanor 50 mg BID group and 314 [21.5%] subjects into the placebo group). A total of 139 (22.4%) subjects withdrew prior to completing the 26-week treatment period, with the most common reasons

for withdrawal being withdrawal of consent (47 [7.6%] subjects), loss to follow-up (31 [5.0%] subjects), and AE (27 [4.4%] subjects). The majority of AEs leading to discontinuation were diarrhea. Other reasons for withdrawal included: two subjects who had moved out of state, one subject got pregnant, one subject no longer met inclusion criterion #10 (daily access to a touch-tone telephone), noncompliance with study diary and multiple missed study visits (two subjects), and unspecified (two subjects).

There was slightly greater overall dropout on tenapanor than placebo (24.2% versus 20.7%), largely attributable to greater dropout due to AE on tenapanor than placebo (7.5% vs. 1.3%).

Table 25 shows the disposition of the subjects for Study 302.

Table 25. Subject Disposition, 12-Week Treatment Period (All Subjects), Study 302

Category	Placebo	Tenapanor 50 mg BID	Overall
Screened – n			1461
Screen failure – n(%)[1]			841 (57.6)
Primary reason for screen failure – n(%)[1]			
Did not meet inclusion/exclusion criteria			150 (10.3)
Did not meet PRO/IVRS diary criteria			665 (45.5)
Withdrawal of consent			22 (1.5)
Lost to follow-up			0
Other			4 (0.3)
Randomized – n(%)[1]	314 (21.5)	306 (20.9)	620 (42.4)
Completed the 26-week treatment period – n(%)[2]	249 (79.3)	232 (75.8)	481 (77.6)
Withdrew prior to completing the 26-week treatment period – n(%)[2]	65 (20.7)	74 (24.2)	139 (22.4)
Primary reason for withdrawal – n(%)[2]			
Adverse event	4 (1.3)	23 (7.5)	27 (4.4)
Unsatisfactory response to study treatment	7 (2.2)	4 (1.3)	11 (1.8)
Non-compliance with study treatment	3 (1.0)	3 (1.0)	6 (1.0)
Decision by Investigator/Sponsor	6 (1.9)	2 (0.7)	8 (1.3)
Withdrawal of consent	26 (8.3)	21 (6.9)	47 (7.6)
Protocol violation	0	1 (0.3)	1 (0.2)
Lost to follow-up	16 (5.1)	15 (4.9)	31 (5.0)
Death	0	0	0
Other	3 (1.0)	5 (1.6)	8 (1.3)
Safety Analysis Set – n(%)[2]	300 (95.5)	293 (95.8)	593 (95.6)
Reasons excluded from Safety Analysis Set – n(%)[2]			
Did not receive any study drug	0	0	0
Serious breach of GCP	14 (4.5)	13 (4.2)	27 (4.4)
ITT Analysis Set – n(%)[2]	300 (95.5)	293 (95.8)	593 (95.6)
Reasons excluded from ITT Analysis Set – n(%)[2]			
Did not meet inclusion/exclusion criteria	0	0	0
Did not receive any study drug	0	0	0
Serious breach of GCP	14 (4.5)	13. (4.2)	27 (4.4)
PP Analysis Set – n(%)[2]	233 (74.2)	228 (74.5)	461 (74.4)
Primary reason excluded from PP Analysis Set – n(%)[2]			
Excluded from ITT Analysis Set	14 (4.5)	13 (4.2)	27 (4.4)
Major protocol deviations	11 (3.5)	13 (4.2)	24 (3.9)
Inadequate daily IVRS diary assessments	46 (14.6)	50 (16.3)	96 (15.5)
Study drug compliance	10 (3.2)	2 (0.7)	12 (1.9)

Abbreviations: BID = twice daily; GCP = Good Clinical Practice; ITT = Intent-to-treat; IVRS = Interactive voice response system; n = Number of subjects analyzed; PP = Per-Protocol; PRO = Patient reported outcome

1 Percentages were calculated using the number of subjects screened as the denominator

2 Percentages were calculated using the number of subjects randomized as the denominator

Source: Applicant's Clinical Study Report Pages 53 and 54/1738

Demographics and Baseline Characteristics

Subject demographics and baseline characteristics are presented in Table 26 below.

Similar to study 301, the demographics of the study were generally reflective of the IBS-C population. The study enrolled a broad population of ages and adequate representation of African American subjects as well as Hispanic subjects. The demographic and baseline characteristics were similar between treatment groups.

Table 26. Demographics and Baseline Characteristics, 12-Week Treatment Period (All Subjects), Study 302

Characteristic	Placebo (N = 300)	Tenapanor 50 mg BID (N = 293)	Overall (N = 593)
Age at informed consent (years)			
n	300	293	593
Mean (SD)	44.8 (13.80)	46.1 (13.10)	45.4 (13.46)
Median	44.0	46.0	45.0
Minimum – Maximum	18 – 75	18 – 74	18 – 75
Gender – n(%)			
Male	53 (17.7)	53 (18.1)	106 (17.9)
Female	247 (82.3)	240 (81.9)	487 (82.1)
Race – n(%)			
White	192 (64.0)	185 (63.1)	377 (63.6)
Black or African American	92 (30.7)	92 (31.4)	184 (31.0)
Asian	9 (3.0)	12 (4.1)	21 (3.5)
American Indian or Alaska Native	0	1 (0.3)	1 (0.2)
Native Hawaiian or Other Pacific Islander	0	0	0
Multiple*	3 (1.0)	1 (0.3)	4 (0.7)
Unknown	0	1 (0.3)	1 (0.2)
Other	4 (1.3)	1 (0.3)	5 (0.8)
Ethnicity – n(%)			
Hispanic or Latino	78 (26.0)	77 (26.3)	155 (26.1)
Not Hispanic or Latino	222 (74.0)	216 (73.7)	438 (73.9)
Baseline weight (kg)			
n	300	293	593
Mean (SD)	84.76 (21.575)	83.38 (20.453)	84.08 (21.022)
Median	80.95	80.50	80.80
Minimum – Maximum	42.1 – 180.9	50.0 – 156.7	42.1 – 180.9
Baseline height (cm)			
n	300	293	593
Mean (SD)	165.47 (8.278)	165.40 (8.948)	165.44 (8.608)
Median	165.05	165.00	165.00
Minimum – Maximum	149.0 – 198.0	137.0 – 193.0	137.0 – 198.0
Baseline BMI (kg/m^2)			
n	300	293	593
Mean (SD)	30.88 (7.271)	30.50 (7.176)	30.69 (7.221)
Median	29.80	29.00	29.60
Minimum – Maximum	17.5 – 64.9	18.7 – 59.2	17.5 – 64.9

Abbreviations: BID = Twice daily; BMI = Body mass index; ITT = Intent-to-treat; N = Number of subjects randomized; n = Number of subjects analyzed; SD = Standard deviation

Note: Baseline for body weight and BMI was defined as the measurement taken at Day 1 pre-dose. If missing, the last measurement prior to the first dose of study drug was used

Source: Applicant's Clinical Study Report Pages 56 and 57/1738

Efficacy Results: Primary Endpoint

A total of 107 (36.5%) subjects in the tenapanor 50 mg BID group and 71 (23.7%) subjects in the placebo group were considered to be 6/12 week overall responders in the ITT population. Compliance was similar between groups.

The treatment difference and the 95% CI in 6/12 week overall responder rates for tenapanor versus placebo was 12.85% (5.54%, 20.17%), which was statistically significant (p<0.001) based on a CMH test stratified by pooled investigator sites (primary analysis). Table 27 summarizes the 6/12 week overall responder rates for the both ITT as well as the PP analysis population.

Table 27. Study 302 Primary Efficacy Endpoint Results, ITT and PP

6/12 Weeks Overall Responder Rate	Tenapanor	Placebo	P-Value	Difference (95% CI)
ITT (primary analysis)	107/293 (36.5%)	71/300 (23.7%)	<0.001	12.9% (5.5%, 20.2%)
PP (exploratory analysis)	99/228 (43.4%)	60/233 (25.8%)	<0.001	17.7% (9.1%, 26.2%)

Abbreviations: CI, confidence interval; ITT, intent-to-treat; PP, per-protocol
Source: TEN-01-302 Clinical Study Report Table 11-6 (pg. 64) and Table 14.2.1.2 (pg. 312). Results were confirmed by FDA.

The findings by the FDA review team confirmed the Applicant's results.

Similar to Study 301, FDA requested an additional sensitivity analysis ("zero imputation method"), of the primary and key secondary endpoints where days with missing data were imputed with zero for the CSBM count for that day. Utilizing this method, 37% of tenapanor treated patients were overall responders for 6/12 weeks vs 23% on placebo, consistent with the results in the ITT population (results in Applicant's response to IR received 4/23/19, TEN-01-302-Table 14.2.1.1s).

Secondary Efficacy Endpoints

The efficacy results for the clinically relevant secondary endpoints are shown in Table 28. The first eight endpoints listed were controlled for multiplicity, while durable overall response was an exploratory endpoint.

Table 28.Study 302 Secondary Endpoints Results, ITT Population

Secondary Endpoints in Order	Tenapanor	Placebo	P-Value	Difference (95% CI)
Overall CSBM response (6/12)	139/293 (47.4%)	100/300 (33.3%)	<0.001	14.1% (6.3%, 21.9%)
Overall abdominal pain response (6/12)	146/293 (49.8%)	115/300 (38.3%)	0.005	11.5% (3.6%, 19.4%)
Overall response (9/12)	54/293 (18.4%)	16/300 (5.3%)	<0.001	13.1% (8.0%, 18.2%)
Overall CSBM Response (9/12)	65/293 (22.2%)	18/300 (6.0%)	<0.001	16.2% (10.7%, 21.7%)
Overall Abdominal Pain Response (9/12)	105/293 (35.8%)	80/300 (26.7%)	0.02	9.2% (1.7%, 16.6%)
Overall Response (13/26)	104/293 (35.5%)	73/300 (24.3%)	0.003	11.2% (3.8%, 18.5%)
Overall CSBM Response (13/26)	121/293 (41.3%)	93/300 (31.0%)	0.01	10.3% (2.6%, 18.0%)
Overall Abdominal Pain Response (13/26)	147/293 (50.2%)	120/300 (40.0%)	0.01	10.2% (2.2%, 18.1%)
*"Durable overall response"	53/293 (18.1%)	15/300 (5.0%)	<0.001	13.1% (8.0%, 18.1%)

Abbreviations: CI, confidence interval; CSBM, complete spontaneous bowel movement; ITT, intent-to-treat
Source: TEN-01-302 Clinical Study Report Table 11-7 (pg. 66), Table 11-8 (pg. 67), Table 11-9 (pg. 69) and Table 14.2.1.34 (pg. 340). Results were confirmed by FDA.
*"Durable overall response" was exploratory endpoint, not subject to multiplicity control.

All secondary efficacy endpoints, included in the multiple testing procedure and summarized above, demonstrated statistically significant results in Study 302. The additional exploratory endpoint of "durable overall response" (exploratory analysis) also showed an effect of tenapanor in comparison to placebo. (b) (4)

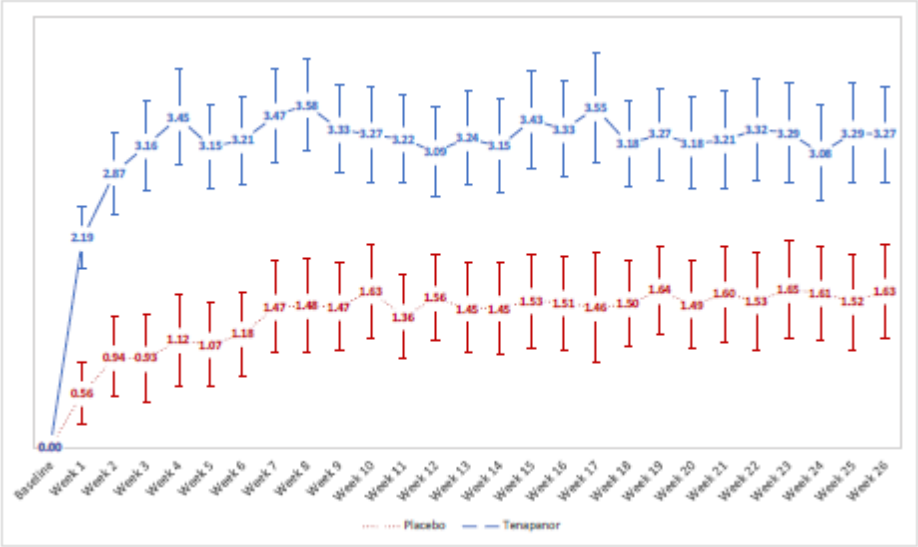
These results were in agreement with that of the FDA review team.

Using the "zero imputation method" described above, the results of the sensitivity analysis to missing data for the secondary endpoints requested by the FDA were consistent with results from the primary analysis of these endpoints (data not shown, Applicant's response to IR dated 4/23/19).

Time to Onset of Clinical Benefit:

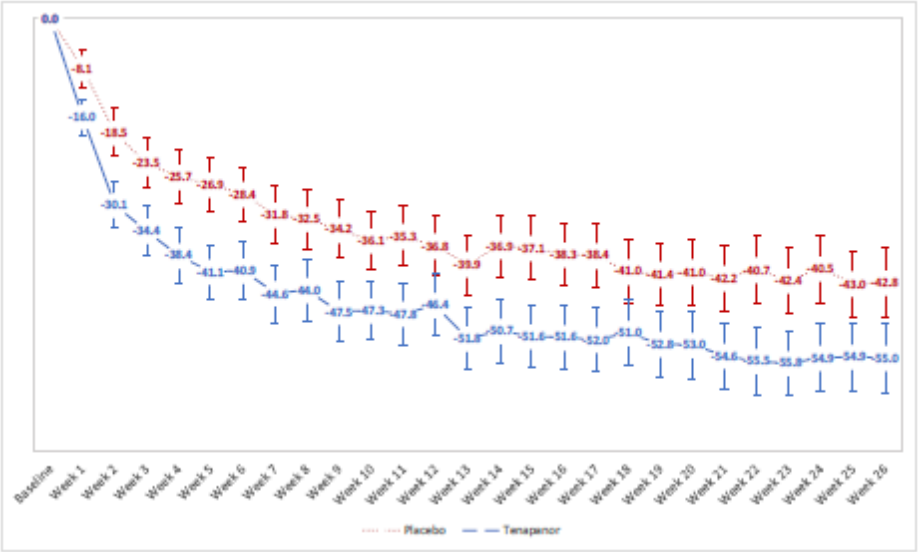
Similarly to Study 301, within Study 302 a pharmacodynamic (PD) effect on both the frequency of CSBMs as well as the abdominal pain scores were noted on average within the first week of treatment.

Figure 6.Least Squares Mean and 95% CI for Change from Baseline in Average Weekly CSBMs (Study 302)- 26 week treatment period



Source: sponsor's response to IR received 8/7/19, ITT population from study 302, refer to CSR Table 14.2.3.1.
Remark: y-axis represents "Average Weekly CSBM"

Figure 7. Least Squares Mean and 95% CI for Percent Change from Baseline in Average Weekly Abdominal Pain Score (Study 302) 26 Week Treatment Period



Source: sponsor's response to IR received 8/7/19, ITT population from study 302, refer to CSR Table 14.2.6.1.
Remark: y-axis represents "Average Weekly Abdominal Pain"

Subgroup Analyses

Table 36 summarizes overall response rates (primary endpoint) by treatment arm and treatment differences with 95% CIs for sex, age category (<65 and ≥65) and race subgroups.

Table 29. Study 302 Subgroup Analyses by Gender, Age Category and Race for Primary Endpoint*

Subgroup	Tenapanor	Placebo	Difference (95% CI)
Gender			
Female	91/240 (37.9%)	62/247 (25.1%)	12.8% (4.6%, 21.0%)
Male	16/53 (30.2%)	9/53 (17.0%)	13.2% (-3.8%, 29.2%)
Age category			
<65	101/271 (37.3%)	65/273 (23.8%)	13.5% (5.8%, 21.1%)
≥65	6/22 (27.3%)	6/27 (22.2%)	5.1% (- 19.3%, 29.4%)
Race			
White	69/185 (37.3%)	47/192 (24.5%)	12.8% (3.6%, 22.1%)
Black	35/92 (38.0%)	22/92 (23.9%)	14.1% (0.9%, 27.3%)
Asian	1/12 (8.3%)	2/9 (22.2%)	-13.9% (-45.2%, 17.5%)

* The primary endpoint was the 6/12 week overall responder rate (CSBM and abdominal pain combined)
Abbreviation: CI, confidence interval
Source: FDA results

No major differences in efficacy were seen by subgroups analyzed in Study 302.
Similarly to Study 301, for all subgroups with the exception of the Asian race subgroup, overall response rates observed in the tenapanor arm were higher than in the placebo arm, although confidence intervals were wide due to the small subgroup sample sizes. The number of patients in the Asian subgroup was very small, precluding definitive conclusions.

Conclusion

Based on the results of the two phase 3 studies, tenapanor demonstrated superiority to placebo for the primary efficacy endpoint of proportion of subjects who were weekly overall responders at 6 or more of the first 12 weeks of double-blind treatment. To be an overall responder for a given week, patients met both the abdominal pain and CSBM response criteria in the same week.

Efficacy results for the multiplicity-controlled secondary endpoints (overall CSBM response for 6 out 12 weeks, overall abdominal pain response for 6 out of 12 weeks, and overall response for 9 out of 12 weeks) further supported primary efficacy findings (statistical significance for all secondary endpoints was achieved in Study 302).

10. Review of Safety

10.1. Safety Review Approach

The safety review of tenapanor focuses primarily on separate analyses of the data from the two adequate and well controlled trials (Study 301 and Study 302) in IBS-C patients. Because of limitations to the safety analysis of data from a randomized withdrawal design (Study 301), the first 12 weeks and last 4 weeks of this trial were analyzed separately. Data on the safety of longer term exposure to tenapanor was obtained by following patients who completed Study 301 or 302 in an open-label extension (Study 303).

Pooled Data for IBS-C

While acknowledging limitations of pooling studies that differ in key elements, we also conducted key analyses in pooled datasets with larger numbers of patients and total duration of exposure as described below. The rationale was to aid in detection of uncommon adverse events. Table 30 below defines three pooled datasets created by the Applicant, based on indication and population studied. For further description of individual studies, please see Section 7.1 above. Pooled datasets from patients being studied for other indications (ESRD, CKD sets) will be discussed later in the review (Section 10.2.12).

Table 30. Safety Review Sets With Dose Ranges

Core Safety Analysis Set	ESRD Safety Analysis Set	Type 2 DM/CKD Safety Analysis Set
D5612C00001 (5 mg BID, 20 mg BID, 50 mg BID, placebo)	(b) (4)	D5610C00001 (5 mg BID, 15 mg BID, 30 mg BID, 60 mg BID, placebo)
RDX 5791-201 (10 mg QD, 30 mg QD, 100 mg QD, placebo)	D5613C00001 (1 mg BID, 3 mg QD, 3 mg BID, 10 mg BID, 30 mg QD, 30 mg BID, placebo)	
TEN-01-301 (50 mg BID)	TEN-02-201 (3 mg BID, 10 mg BID, 15 mg BID, 20 mg BID, 30 mg BID)	
TEN-01-302 (50 mg BID)		
TEN-01-303 (50 mg BID)		

Abbreviations: BID, twice daily; CKD, chronic kidney disease; DM, diabetes mellitus; ESRD, end-stage renal disease; QD, once daily
Source: Reviewer table, adapted from Applicant's Tables 2-3, 2-4 and 2-5, Integrated Summary of Safety (ISS), Sept. 28, 2018, pp. 40–41

Table 31 below defines the safety analysis populations utilized for our analyses and that of the Applicant, with the rationale and limitations of each population. Where a pooled analysis dataset encompassing the combined phase 2 and phase 3 data is required for analysis of adverse events, our Integrated Safety Analysis Set, referred to hereafter as "Integrated Safety

Analysis Set," is utilized. Similarly, where a pooled dataset is needed to assess laboratory parameters, our Laboratory Analysis Set is utilized.

Table 31. Description of Safety Analysis Populations

Population	Tenapanor N Included	Placebo N Included
Study 301 (first 12 weeks) All randomized patients who received at least one blinded treatment	309	301
Study 301 (last 4 weeks) All patients who completed Week 12 and were rerandomized into the randomized-withdrawal (RW) period and received at least one dose of blinded treatment after Week 12	386	129
Study 302 All randomized patients who received at least one dose of study drug	293	300
Applicant's "Core Safety Analysis Set" Patients enrolled in phase 2 or phase 3 studies, excluding site 284 Summarized by group to which patients were initially randomized	1007 (excludes site 284 n=23)	738 (excludes site 284 n=22)

Population	Tenapanor N Included	Placebo N Included
Applicant's "Core Analysis Set" -Includes all randomized patients across phase 2 and 3 studies -Categorized by whether or not patients ever received a dose of tenapanor as follows: 344 patients were counted twice (once in placebo and once in tenapanor) because they received placebo in either the first 12 weeks of Study 301 or the first 26 weeks of 302, and subsequently received at least one dose of tenapanor thereafter, either in RW period of 301, or as active treatment in extension study 303. The purpose was to ensure a "safety population" that does not miss anyone who received at least one dose of tenapanor, regardless of when in study participation it was received. Because the Integrated Safety Analysis Set contains pooled data from trials of different duration and dosing as well as data from an open-label uncontrolled extension trial, analyses using this set are subject to potential bias and should be interpreted with caution. For example, inclusion of lower doses than 50 mg BID in the tenapanor arm may result in underestimates of the risk of the proposed dose, differences in incidence proportions between arms could be due to differences in duration of follow-up (rather than drug effects), and crude pooling of studies with different treatment arms/randomization ratios induce bias (e.g., Simpson's Paradox). In order to mitigate some of the potential bias introduced by differing trial duration, some analyses were conducted using incidence rates (per 100 person-years), not only incidence proportions. Another limitation of the Applicant's Core Analysis Set is that the denominator of the treatment arm is increased , but the denominator for placebo was not increased comparably (patients who initially got tenapanor in Study 301, followed by placebo in RW period, did not contribute an additional patient to the N of the placebo group). This may potentially result in the proportion for a given AE appearing falsely lowered in the treatment arm as compared to placebo. Because of the limitations of the integrated analyses that were conducted, analysis of individual controlled trials is preferred for reporting incidence of specific AEs. However, the use of this set still permits potential detection of rare AEs that would require a larger population exposed to detect. This set does not exclude the GCP violation.	1374 (derived from 1007 from above + 23 from site 284 + 344 who were initially in placebo arm)	760 (derived from 738 above + 22 from site 284)

Population	Tenapanor N Included	Placebo N Included
FDA's Integrated Safety Analysis Set Defined similarly to Applicant's "Core Analysis Set" but excluding patients from Site 284.	1343 (1374 above–31 records of patients who got tenapanor from site 284 (this 31 is comprised of records from 23 individuals who got tenapanor in the first 12 weeks and eight individuals who got placebo in the first 12 weeks followed by tenapanor in the RW period))	738
FDA's Laboratory Analysis Set Includes patients who had baseline and at least one evaluable postbaseline value for the laboratory test in question. For Study 301 subjects, each subject contributed once to the population that they were randomized to, and a second time to the population they were rerandomized to at Week 12. For example, a patient who received placebo in first 12 weeks, and then was re-randomized to active treatment contributes once to each denominator (tenapanor and placebo). Similarly, a patient who got active treatment for initial 12 weeks, and then was rerandomized to active treatment in RW period contributes twice to the tenapanor denominator. This decision was made to avoid one of the limitations of the applicant's "Core Analysis Sets" as described above.	Variable (by available data for given lab test)	Variable (by available data for given lab test)

Abbreviations: AE, adverse event; GCP, good clinical practice; RW, randomized-withdrawal
Source: reviewer's table

Pooled Data for Other Indications

As a supplemental safety analysis, data from all phase 2 and phase 3 trials conducted in ESRD and a single trial in type 2 diabetes with chronic kidney disease (CKD) were analyzed. These results will be discussed in Section 10.2.12, Specific Safety Studies/Clinical Trials in Special Populations. These analyses were performed because post-marketing experience with other IBS drugs such as tegaserod and alosetron supports the utility of examining an older population with comorbidities in the premarketing phase to identify potential safety issues in special populations.

10.1.1. Overall Exposure

A total of 2085 subjects have received tenapanor in clinical trials: 1343 had IBS-C, 412 had ESRD, 77 had type 2 diabetes with CKD, and 253 were healthy volunteers (See Section 7.1). The

total exposure in person-years is 466.1 for IBS-C (of which 422 is at the proposed dosage of 50 mg BID). In the IBS-C program, 312 patients were enrolled in the 52-week, open-label, extension trial, of whom 262 completed the trial. A total of 306 patients were enrolled in the tenapanor 50 mg BID arm in the 26-week, phase 3, controlled trial. Thus, the IBS-C program met the International Conference on Harmonisation E1A[4] recommended targets of approximately 1500 patients exposed to study drug, with more than 300 exposed for 26 weeks and over 100 exposed for 1 year.

Overall, there appears to be adequate exposure to tenapanor in the IBS-C program for the evaluation of common AEs and events with short latency. However, the evaluation of rare or infrequent AEs and/or those that may occur after a long duration of tenapanor exposure may not be adequately assessed in this program.

10.1.2. Demographics / Adequacy of the Safety Population

The demographics of the enrolled population in each of the two phase 3 trials were summarized and describe above in section 9.1.1 and 9.1.2 (Table 21, Table 20 and Table 25, Table 26) and representation was mostly adequate to describe the safety of tenapanor in a broad population. Limited numbers of patients of races other that Caucasian or African American / Black is one notable exception.

Similarly, the Integrated Safety Analysis Data Set appears adequate in terms of total numbers of patients, duration of exposure and diversity (Table 32). The preponderance of female patients in the trials (83%) reflects the known female preponderance of IBS-C patients. The percentage of Asians enrolled in the trials (2%) underrepresents the estimated U.S. Asian population of 5.6%. Only 7% of patients in the Integrated Safety Analysis safety population were over age 65 compared to the estimated 16% of the U.S. population aged 65 and over in 2017. Higher representation of older and male patients in the ESRD and CKD clinical trials provides additional data on potential risks in these populations (See Section 10.2.12 on Specific Safety Studies/Clinical Trials in Special Populations).

[4] See the guidance for industry *The Extent of Population Exposure to Assess Clinical Safety: For Drugs Intended for Long-term Treatment of Non-Life-Threatening Conditions* (March 1995). https://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

Table 32. Demographic Characteristics of FDA's Integrated Safety Analysis Population

Integrated Safety Analysis Database	Race	Age	Sex	Ethnicity
IBS-C on Tenapanor N=1343	White: 916 (68%) Black: 371 (28%) Asian: 33 (2%) Native American: 3 (0.22%) Other, Unknown or Multiple: 20 (1.5%)	≤65 years: 1251 (93%) >65 years: 92 (7%)	Female: 1116 (83%) Male: 227 (17%)	Hispanic or Latino: 377 (28%) Non-Hispanic: 966 (72%)
Placebo N=738	White: 479 (65%) Black: 226 (31%) Asian: 15 (2%) Native American: 2 (0.27%) Other, Unknown or Multiple: 16 (2.2%)	≤65 years: 683 (92%) >65 years: 55 (8%)	Female: 614 (83%) Male: 124 (17%)	Hispanic or Latino: 193 (26%) 545 (74%)

Abbreviations: IBS-C, irritable bowel syndrome with constipation
Source: Reviewer's table, adapted from Applicant's Table 2.1, 2.2 and 2.3, ISS, pp. 19-30, submitted September 12, 2018

10.1.3. Categorization of Adverse Events

The general, categorization of the AEs was appropriate.

AEs were categorized by system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA). All collected data (medical history terms, TEAE, concomitant medications) were coded in the integrated datasets using the MedDRA dictionary version 20.1 and the World Health Organization Drug Dictionary, September 2017 version. Prior to creation of the integrated datasets, MedDRA versions 14.0, 16.0, 18.1 were used for individual studies.

The Applicant defined an AE as follows: "any untoward medical occurrence in a subject administered a pharmaceutical product, whether or not the occurrence has causal relationship with this treatment… This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of the investigational product."

Treatment-emergent adverse events were defined as any of the following:

- Any adverse event that occurs after administration of the first dose of study drug and up through the final visit
- Any event that is considered drug-related regardless of the start date
- Any event that is present at baseline but worsens in severity or is subsequently considered drug-related by the investigator

Categorization of severity of AEs by the Investigator was as follows:

- Mild-- does not interfere in a significant manner with the subject's normal functioning level
- Moderate-- produces some impairment of functioning but is not hazardous to health
- Severe-- produces significant impairment or incapacitation and is a definite hazard to the subject's health

An unexpected adverse experience was defined as any adverse drug experience, the specificity or severity of which is not consistent with the current Investigator's Brochure or available risk information described in the general investigational plan or elsewhere in the current application. Causality of AEs was assigned by the Investigator as follows:

- Not related-- most likely produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs and does not follow a known response pattern to the study drug
- Possibly related-- follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study drug but could have been produced by other factors
- Probably related-- follows a reasonable temporal sequence from the time of drug administration, and/or follow a known response pattern to the study drug and cannot be reasonably explained by other factors.

Serious AEs (SAEs) were defined consistent with 21 CFR 312.32.

For Study 301, AEs were queried at each visit through the last visit at Week 16 (the end of the randomized withdrawal period). For Study 302, AEs were queried at each visit through the last visit at Week 26 (the last dose of study drug). For Study 303, AEs were queried at each visit, and at intervening telephone visits. All SAEs were followed until satisfactory resolution or clear determination that a subject's AE was due to a stable or chronic condition or concomitant illness.

To better detect potential safety signals, we recoded the following preferred terms as shown in the Table 33 below.

Table 33. Preferred Terms Used in Safety Signal Analysis

Preferred Term	Included Terms
Rectal bleeding	Rectal hemorrhage, hemorrhoidal hemorrhage, hematochezia, hemorrhagic diarrhea, lower gastrointestinal hemorrhage
Chest pain	Chest pain, chest discomfort, noncardiac chest pain, angina pectoris
Ischemic/thromboembolic events	Myocardial infarction, coronary artery stenosis, coronary artery occlusion, ischemic stroke, myocardial ischemia, pulmonary embolism, peripheral ischemia, deep vein thrombosis, peripheral artery occlusion, peripheral artery ischemia, venous occlusion, subclavian artery occlusion, shunt thrombosis, arteriovenous (AV) fistula thrombosis, AV fistula stenosis
Abdominal surgical emergencies	Emergent surgery due to small intestinal obstruction, cholecystitis, appendicitis, endometriosis, ovarian vein thrombosis and diverticulitis
Acute kidney injury and/or dehydration	Acute kidney injury, blood creatinine increased, azotemia, blood urea increased, glomerular filtration rate decreased, dehydration
Abdominal pain	Abdominal pain upper, abdominal pain lower, abdominal pain right-sided, abdominal pain left-sided and abdominal discomfort

Source: Reviewer's table

Appropriate safety evaluations were performed as a part of the drug development program. The safety assessments consisted of:

- Monitoring of all spontaneous AEs and SAEs with their severity and relationships to

study drug and pregnancies

- Hematology, blood chemistry, urinalysis, pregnancy testing, vital signs, physical examinations, ECGs.

The evaluation of safety was conducted during visits to the clinic and all patients entered daily diary entry data. Schedule of events is provided in the Clinical Appendices, Section 16.1.

10.2. Safety Result

10.2.1. Overall Rates of Adverse Events

Overall, rates of TEAEs were higher on tenapanor than placebo, and AEs leading to discontinuation occurred with greater frequency on tenapanor than placebo. SAEs occurred rarely and in comparable proportion of patients on tenapanor or placebo. Table 34 below summarizes rates of AEs across the controlled portions of Study 301 and Study 302.

Table 34. Rates of Adverse Events in Phase 3 Trials

Number of patients with	Study 301* Tenapanor N=309 n (%)	Study 301* Placebo N=301 n (%)	Difference in Proportions (%) (95% CI)	Study 302 Tenapanor N=293 n (%)	Study 302 Placebo N=300 n (%)	Difference in Proportions (%) (95% CI)
Any TEAE	110 (36)	74 (25)	**11 (4, 18)**	143 (49)	124 (41)	**7 (-0.01, 15)**
Any SAE	4 (1.3)	0 (0)	**1, (-0.2, 3)**	4 (1.4)	6 (2.0)	**-0.6 (-2, 3)**
Any TEAE leading to discontinuation	23 (7.4)	2 (0.7)	**7 (4, 10)**	23 (7.8)	3 (1.0)	**7 (4, 11)**
Any TEAE leading to death	0 (0)	0 (0)	**0 (-1, 1)**	0 (0)	0 (0)	**0 (-1, 1)**

* First 12 weeks of Study 301 only, using ITT population as denominator
Source: Reviewer's table, created from ADAE datasets for study 301 and 302.

10.2.2. Dropouts and/or Discontinuations Due to Adverse Effects

As seen in Table 34 above, in both the phase 3 trials, more patients on tenapanor discontinued the study due to AEs than patients on placebo. In total (both trials combined), AEs resulting in discontinuation occurred in 46/602 (7.6%) of patients receiving tenapanor and 5/601 (0.8%) of patients receiving placebo. Diarrhea was the most common AE leading to discontinuation.

There appeared to be a dose-response relationship between tenapanor dose and frequency of diarrhea in the FDA's Integrated Safety Analysis cohort (Table 35). Diarrhea is an expected adverse event for this drug based on the mechanism of action.

Table 35. Diarrhea AEs Leading to Discontinuation by Treatment Group and Dosage, Integrated Safety Analysis Data Set

AE Diagnosis (SOC and PT)	Number of AEs/Total Number of Patients per Dose	Percentage of Patients	Dosage
Placebo	4/738	0.54%	Placebo
Tenapanor	2/87	2.3%	5 mg BID
	3/87	3.4%	20 mg BID
	64/675	9.5%	50 mg BID
	69/1343	5.1%	All doses

Abbreviations: AE, adverse event; BID, twice daily; PT, preferred term; SOC, system organ class
Source: FDA's table, adapted from Applicant's Table 24, ISS, , Studies D5612C00001, RDX5791-201, TEN-01-301, TEN-01-302, submitted September 12, 2018

10.2.3. Deaths

No IBS-C patients died during the development program. Two patients died in the ESRD trials, see Section 10.2.12 and narratives in Appendix 16.1.1. Although deemed by the investigators to be unrelated to the study drug, these deaths involved the occurrence of rare adverse events seen in other patients on tenapanor, and are therefore considered potentially relevant to patients with IBS-C.

10.2.4. Serious Adverse Events

In the controlled portions of Study 301 and Study 302, there were few SAEs reported. In the first 12 weeks of Study 301, there were 5/307 (1.6%) SAEs on treatment and 0/299 (0%) on placebo. Of the five SAEs on tenapanor, three were psychiatric (alcohol withdrawal, panic attack and major depression), one was neurologic (migraine), and one was osteoarthritis. In Study 302, there were 4/293 (1.4%) SAEs on treatment and 7/300 (2.3%) on placebo. None of the SAEs on tenapanor in Study 302 was neuropsychiatric. Instead, the 4 SAEs in Study 302 were primarily gastrointestinal (nausea, diarrhea, abdominal pain and chronic obstructive pulmonary disease exacerbation).

Due to low numbers of serious adverse events in the controlled trials, we also assessed SAEs in the Integrated Safety Analysis Set Analysis Set (which provides a larger database) to determine if any particular SOC was over-represented. Results were generally comparable with findings from the controlled trials. Details are described in the Clinical Appendix: 16.1.3. including Table 60.

10.2.5. Common Adverse Events

Diarrhea was the most common TEAE in tenapanor-treated patients for all safety analysis subsets. To increase the sensitivity of the detection of TEAEs other than diarrhea and determine if other events should be included in the label as common AEs, we analyzed TEAEs that occurred in at least 1% of tenapanor-treated patients and for which the risk difference with placebo exceeded 1%. The following analyses justify inclusion of the following additional terms in the prescribing information as "common adverse events": abdominal distension, abnormal gastrointestinal sounds, flatulence, dizziness.

Results for common TEAEs for the two phase 3 trials, Study 301 and Study 302, for IBS-C were analyzed separately, as the duration of treatment differed. TEN-01-301 consisted of a 12-week, randomized, double-blind, placebo-controlled trial, followed by a 4-week rerandomized, withdrawal period. These two periods were also analyzed separately.

Study 301 (First 12 Weeks)

In Study 301, the most commonly reported AE was diarrhea, occurring in 45/309 (15%) of tenapanor patients and 5/301 (1.7%) of placebo treated patients (Table 36). Two additional gastrointestinal TEAEs, abdominal distension and abnormal gastrointestinal sounds, also occurred in greater than 1% of tenapanor-treated patients with a risk difference of greater than 1% versus placebo.

Table 36. Common Treatment-Emergent Adverse Events With Risk Difference >1% Between Tenapanor and Placebo: Study 301, First 12 Weeks

Diagnosis	Tenapanor N=309	Placebo N=301	Risk Difference (%, 95% CI)
Diarrhea	45 (15%)	5 (1.7%)	13 (9,17)
Abdominal distension	5 (1.6%)	0 (0%)	1.6 (0.6,2.6)
Gastrointestinal sounds abnormal	4 (1.3%)	1 (0.33%)	1 (0,2)

Source: Reviewer's table, derived from Applicant's ADAE electronic ADAM data set for study TEN-01-301, submitted September 12, 2018, analyzed with JMP 13.0 software

Study 301 (Last 4 Weeks/Randomized Withdrawal Period)

In this safety population (Table 37), diarrhea was still the most common TEAE, reported in 8.3% of tenapanor-treated patients compared to 0% of placebo-treated patients. In addition, nausea and vomiting occurred at slightly higher rates (1.8%) in tenapanor-treated patients as compared to placebo-treated patients (0.8%). The clinical significance of nausea and vomiting (which were not present during the 12 week, controlled period, of Study 301, nor in the 26 week controlled period of Study 302) is unclear. This finding may be due to chance and the risk difference is small, so a decision was made not to include nausea and vomiting in Section 6 of the label as common AEs.

Table 37. Common TEAEs with Risk Difference >1% between Tenapanor and Placebo: Study 301, Last 4 Weeks

Diagnosis	Tenapanor N=386 patients	Placebo N=129 patients	Risk Difference (%, 95% CI)
Diarrhea	32 (8.3%)	0 (0%)	8.3 (5.3,11.3)
Nausea and/or vomiting	7 (1.8%)	1(0.8%)	1 (-1,3)

Abbreviation: TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from Applicant's Adverse Event line listings for Study TEN-01-301t, submitted September 12, 2018, analyzed with JMP 13.0 software

Study 302

In this 26-week, phase 3 trial, rates of TEAEs were similar to those reported for Study 301, the 12-week, phase 3 trial (Table 38). Cumulative rates of diarrhea were 16% in tenapanor-treated patients and 3.7% in placebo-treated patients. Less common gastrointestinal TEAEs included abdominal distension (3.4% tenapanor versus 0.33% placebo), flatulence (3.1% tenapanor versus 1% placebo) and abnormal gastrointestinal sounds (1% tenapanor versus 0% placebo). Dizziness was the only non-gastrointestinal TEAE found in 1.7% of tenapanor-treated patients

and 0.67% of placebo-treated patients. It is interesting that abdominal distension occurred in more than 3% of patients in Study 302; published reports in the literature note that some patients reported an improvement in bloating on this medication (Black et al. 2018). Different subsets of patients may exist, some of whom derive improvement in bloating on the medication and some of whom experience worsening abdominal distension.

Table 38. Common TEAEs with Risk Difference >1 between Tenapanor and Placebo: Study 302

Diagnosis	Tenapanor N=293	Placebo N=300	Risk Difference (%, 95% CI)
Diarrhea	47 (16%)	11 (3.7%)	12 (7,17)
Abdominal distension	10 (3.4%)	1 (0.33%)	3 (1,5)
Flatulence	9 (3.1%)	3 (1%)	2.1 (0.1,4.1)
Dizziness	5 (1.7%)	2 (0.67%)	1 (-1,3)
Gastrointestinal sounds abnormal	3 (1.0%)	0 (0%)	1 (0,2)

Abbreviation: TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from Applicant's ADAE electronic ADAM data set, submitted September 12, 2018, analyzed with JMP 13.0 software

10.2.6. Adverse Events of "Severe" Intensity

In the controlled portions of Study 301 and Study 302, small numbers of AEs graded by investigators as "severe" in intensity occurred. In the first 12 weeks of Study 301, 13/307 (4.2%) severe AEs occurred on tenapanor treatment and 2/299 (0.66%) on placebo. Of the severe AEs that occurred on tenapanor in Study 301, 10/13 were gastrointestinal (9 diarrhea). Similarly, in Study 302, 12/293 (4.1%) severe AEs occurred on tenapanor treatment and nine (3.0%) on placebo. Of the severe AEs on tenapanor in this study, 10/12 were gastrointestinal (7 diarrhea). Taken together, severe diarrhea was reported in 2.5% of patients across the controlled portions of the two trials.

Due to low numbers of severe adverse events in the controlled trials, severe AEs were also assessed within the Integrated Safety Analysis Set (a larger database). The trends were similar, and detailed data are presented within the clinical appendix, Section 16.1.3 (Table 61) below.

10.2.7. Laboratory Findings

General Approach

Comparisons between patients on tenapanor and placebo were independently conducted by the FDA for the following laboratory values: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin (BILI), bicarbonate, chloride, hemoglobin, leukocytes, neutrophils, platelet count, potassium, and sodium. Mean laboratory values at each visit and mean change from baseline values were compared between treatment groups for the Integrated Safety Analysis Set and for the individual phase 3 trials. No significant differences between treatment groups for any of the laboratory tests analyzed were detected (data not shown).

For laboratory values of special interest, liver function tests and potassium, further analyses were conducted using shift tables and are presented below. Potassium level was analyzed separately due to findings of hyperkalemia in some ESRD and CKD patients treated with tenapanor (see Section 10.2.12, Specific Safety Studies/Clinical Trials in Special Populations). Bicarbonate was also analyzed due to the potential for dehydration and resultant acidosis in patients with diarrhea, but data are not shown here due to absence of significant findings.

Liver Function Tests

Evaluation for potential drug-induced liver injury (DILI) was conducted. The data do not suggest the drug has a high likelihood for causing significant DILI. Specifically, there were no patients who met Hy's law criteria (rise in ALT or AST >3X the upper limit of normal [ULN] with accompanying risk in total bilirubin >2X ULN).

Within the controlled portions of study 301 and study 302, each analyzed separately, the data do not show an increased rate of experiencing ALT elevation associated with use of tenapanor over 12 or 26 weeks.

Table 39. Shift in ALT, Controlled Portions of Study 301 and 302

	Study 301*		Study 302	
	Tenapanor	Placebo	Tenapanor	Placebo
Shift from Normal to High ALT (>55 U/L)	7/295 (2.4%)	10/288 (3.5%)	10/276 (3.6%)	13/284 (4.6%)

Source: reviewer's analysis of ADLB datasets for Trials 301 and 302, and applicants response to labeling IR received 9/6/19.
* Limited to first 12 weeks

There were very small numbers of patients with pre-existing baseline elevation of ALT, but including them in the analysis above did not change the overall conclusion (data not shown).

Additional evaluation for a potential DILI signal was conducted to assess whether more ALT or AST elevations >2X ULN were present in tenapanor-treated patients compared with placebo, and whether a similar trend was seen with greater degrees of AST/ALT elevation (i.e., >5X or >10X ULN).

Shifts in Transaminases From Baseline in the Two Phase 3 Studies

Separate analysis of Study 301 (including RW period and extension) and Study 302 (including extension) showed that a slightly greater incidence proportion and incidence rate of patients on treatment than placebo experienced elevation of ALT and AST in Study 302 (Table 40, Table 41, Table 43); a similar trend was not seen in Study 301 (Table 40, Table 42). The reason for this is unclear, but may be a chance finding. In both Studies 301 and 302, transaminases were obtained at baseline and every 4 weeks thereafter through 12 weeks. In Study 302, they were also assessed at 16 weeks, 20 weeks and 26 weeks. In Study 303, the extension trial, they were assessed at 26 and 39 weeks.

Table 40. Abnormal Alanine Aminotransferase Shifts From Baseline, Study 301 (Including Randomized Withdrawal Period and Safety Extension)

	Treatment Arm	
Highest Postrandomization Value	**Tenapanor (N=317 Patients*) *203 PY***	**Placebo (N=306 Patients*) *87 PY***
Normal ALT at baseline	N=308 normal ALT baseline values	N=299 normal ALT baseline values
ALT >2X ULN, ≤3X ULN	0	0
ALT >3X ULN, ≤5X ULN	0	0
ALT >5X ULN, ≤10X ULN	0	0
ALT >10X ULN, ≤15X ULN	0	0
Abnormal ALT at baseline^	N=9 abnormal baseline ALT values	N=7 abnormal baseline ALT values
ALT >2X ULN, ≤3X ULN	2 (22%)	0
ALT >3X ULN, ≤5X ULN	0	0
ALT >5X ULN, ≤10X ULN	0	0
ALT >10X ULN, ≤15X ULN	0	0
Total ALT elevation	2 (0.63%) *0.99/100 PY*	0 *0/100 PY*

* Total number of patients for whom evaluable data was available
^ includes only patients whose postbaseline value was higher than baseline abnormal.
Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal
Source: FDA's table, derived from Applicant's LB STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

Table 41. Abnormal Alanine Aminotransferase Shifts From Baseline, Study 302 (Including Safety Extension)

	Treatment Arm	
Highest Postrandomization Value	**Tenapanor (N=311 Patients*) *187 PY***	**Placebo (N=312 Patients*) *132 PY***
Normal ALT at baseline	N=304 normal ALT baseline values	N=305 normal ALT baseline values
ALT >2X ULN, ≤3X ULN	1 (0.33%)	0
ALT >3X ULN, ≤5X ULN	2 (0.67%)	0
ALT >5X ULN, ≤10X ULN	0	0
ALT >10X ULN, ≤15X ULN	0	0
Abnormal ALT at Baseline^	N=7 abnormal baseline ALT values	N=7 abnormal baseline ALT values
ALT >2X ULN, ≤3X ULN	2 (29%)	1 (14%)
ALT >3X ULN, ≤5X ULN	0	0
ALT >5X ULN, ≤10X ULN	0	0
ALT >10X ULN, ≤15X ULN	0	0
Total ALT elevation	5 (1.6%) *2.7/100 PY*	1 (0.32%) *0.76/100 PY*

* Total number of patients for whom evaluable data was available
^ includes only patients whose postbaseline value was higher than baseline abnormal.
Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal
Source: FDA's table, derived from Applicant's LB STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

Table 42. Abnormal Aspartate Aminotransferase Shifts From Baseline, Study 301 (Including Randomized Withdrawal Period and Safety Extension)

	Treatment Arm	
Highest Postrandomization Value	**Tenapanor (N=317)* *203 PY***	**Placebo (N=306*) *87 PY***
Normal AST at baseline	N=301	N=289
AST >2X ULN, ≤3X ULN	1 (0.33%)	1 (0.35%)
AST >3X ULN, ≤5X ULN	1 (0.33%)	0
AST >5X ULN, ≤10X ULN	1 (0.33%)	2 (0.70%)
AST >10X ULN, ≤15X ULN	0	0
Abnormal AST at baseline^	N=16	N=17
AST >2X ULN, ≤3X ULN	3 (19%)	2 (12%)
AST >3X ULN, ≤5X ULN	1 (6.2%)	0
AST >5X ULN, ≤10X ULN		0
AST >10X ULN, ≤15X ULN	0	0
Total AST elevation	7 (2.2%)	5 (1.6%)
	3.4/100 PY	*5.7/100 PY*

* Total number of patients for whom evaluable data was available.
^ includes only patients whose postbaseline value was higher than baseline abnormal.
Abbreviations: AST, aspartate aminotransferase; ULN, upper limit of normal
Source: FDA's table, derived from Applicant's LB STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

Table 43. Abnormal Aspartate Aminotransferase Shifts From Baseline, Study 302 (Including Safety Extension)

	Treatment Arm	
Highest Postrandomization Value	**Tenapanor (N=304*) *187 PY***	**Placebo (N=312*) *132 PY***
Normal AST at baseline	N=288	N=300
AST >2X ULN, ≤3X ULN	2 (0.69%)	2 0.67%)
AST >3X ULN, ≤5X ULN	2 (0.69%)	0
AST >5X ULN, ≤10X ULN	0	
AST >10X ULN, ≤15X ULN	0	0
Abnormal AST at baseline^	N=16	N=12
AST >2X ULN, ≤3X ULN	5 (31%)	2 (17%)
AST >3X ULN, ≤5X ULN		0
AST >5X ULN, ≤10X ULN	1 (6.2%)	0
AST >10X ULN, ≤15X ULN	0	0
Total AST elevation	10 (3.3%)	4 (1.3%)
	5.3/100 PY	*3.0/100 PY*

* Total number of patients for whom evaluable data was available
^ includes only patients whose postbaseline value was higher than baseline abnormal.
Abbreviations: AST, aspartate aminotransferase; ULN, upper limit of normal
Source: FDA's table, derived from Applicant's LB STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

No clear temporal pattern was noted in the onset of transaminase elevations in either of the two controlled phase 3 trials. The time to onset ranged from 1 to 378 days after initiation of the study drug with a median time to onset of 85 days. Details of cases in which the ALT or AST was more than 2X ULN are provided in the subsection, Individual Case Listing of Patients with Elevated Transaminases in the Integrated Safety Analysis Set, below. In most cases the elevations of transaminases were either likely related to concomitant medications or intercurrent events, or resolved or were improving while on treatment without intervention or study drug discontinuation, thereby not suggesting a strong signal of drug induced liver injury.

There were rare cases where either there was no other likely explanation for the rise in liver enzyme(s) or where additional evaluation or longer term follow-up was not provided, and therefore a causal relationship to tenapanor could not be excluded. There were no cases of serious liver injury reported. The label will reflect the potential for tenapanor to cause elevations of AST and ALT.

Liver Function Tests in the Integrated Safety Analysis Set

Similar to the findings when the phase 3 trials were analyzed separately, within the Integrated Safety Analysis Set there was limited evidence of drug-induced liver injury. The details of this analysis and supporting tables are shown in the Clinical Appendix, Section 16.1.3 (Table 62, Table 63, Table 64). An almost 5-fold increase occurred in the percentage of patients with postbaseline ALT elevations in tenapanor- versus placebo-treated patients (Table 62) but the numbers were very small (0.52 versus 0.11%, 1.8/100 PY vs 0.4/100 PY). Of note, most of these shifts were less than 3 times the upper limit of normal and occurred in patients who had abnormal ALT at baseline. No significant shifts in alkaline phosphatase occurred in either group (data not shown).

Narratives of Patients with Elevated Transaminases from the Integrated Safety Analysis Set

Nine patients (eight on tenapanor, one placebo) in the Integrated Safety Analysis Set had an elevated AST or ALT >2X ULN. Additional information on these patients and their evaluation and outcomes were requested in an Information Request on February 25, 2019. A summary of this information appears below in Table 44. Of the patients with laboratory values that suggest DILI, the we identified only one case for which DILI could not be excluded according to the guidance for industry *Drug-Induced Liver Injury: Premarketing Clinical Evaluation* (July 2009). That patient had a favorable outcome, which is reassuring.

The one case in question was patient TEN-01-302-(b) (6) (row with * in table below), who had normal baseline LFTs, and postbaseline elevations that peaked at 2.6 and 3.1X ULN for ALT and AST, respectively. This patient was a 52-year-old white male with a BMI greater than 30 and multiple co-morbidities (diabetes, asthma, hypertension, hyperlipidemia on statins). He withdrew from the study at Day 78 due to serious adverse event of abdominal pain (which could potentially have been related to hepatitis, as cause of the pain was not identified in the documentation available). His bilirubin remained normal throughout. After discontinuation of therapy, his liver enzymes normalized, and the event was reported as “resolved.” Given the timing and resolution upon discontinuation, we could not exclude the contribution of tenapanor to this AE.

An additional four cases of elevated AST or ALT >2X ULN (TEN-01-301-(b) (6), TEN-01-302-(b) (6), TEN-01-302-(b) (6), TEN-01-302-(b) (6) in Table 44) were deemed possibly related to study drug because one or more laboratory abnormalities were temporally related to study drug, did not resolve on study drug and could not be explained by the introduction of a new co-medication. Because no follow-up occurred, it is not possible to determine the relationship with

certainty. Reassuringly, none of these patients had evidence of significant impairment of hepatic function, nor serious events reported in association with these laboratory findings.

Of the nine cases of ALT elevation, eight of nine patients had elevated BMI and eight of nine patients had exposure to concomitant medications that are reported to cause DILI in the literature. All nine cases occurred at doses of 50 mg BID, the proposed marketing dose and the highest dose tested in the combined trials. No clear trend was noted in the temporal occurrence of the transaminase elevations.

Table 44. Cases of ALT Shifts by Demographics, BMI, Visit, Concomitant Medications and Outcome, Integrated Safety Analysis Data Set

Patient and Study Number	Demo-graphics	Arm	Shift ALT (X ULN)	Shift AST (X ULN)	Max Post-baseline TBili (X ULN)	BMI	Visit (V) and Day (D)	Concomitant Meds	Outcome and Assessment of DILI Likelihood
TEN-01-301-(b) (6)	35HF	T	1.5 to 2.8	1.4 to 3.9	0.6	>30	ET/V4 D378	Ibuprofen; MVI; Esomeprazole	Unknown outcome **Possible**
TEN-01-302-(b) (6)	35HF	T	0.5 to 3.1	0.7 to 3.3	0.5	>30	V9 D183	None	Unknown outcome **Possible**
TEN-01-302-(b) (6)	55WF	T	0.7 to 4.8	1.2 to 7.4	0.3	>30	V4 D29	Clonazepam; fluoxetine; quetiapine; vitamin D; salbutamol; loratadine; baclofen; ketaconazole shampoo (D 116); paracetamol; meloxicam; estradiol; sennoside; Vicodin; omeprazole; atorvastatin; ondansetron; phentermine; Axotal; paracetamol; cefazolin; docusate sodium; enoxaparin; famotidine; fluticasone nasal spray; gabapentin; naproxen; oxycodone; quetiapine	Resolved on treatment **Unlikely**
TEN-01-302-(b) (6)	52WM	T	0.6 to 2.6	0.7 to 3.1	0.6	>30	ET D76 (withdrew at D78 due to abdominal pain. hospitalized)	Doxepin; aspirin; clonazepam; ziprasidone; allopurinol; amlodipine; Byetta; doxazosin; Invokana; isosorbide mononitrate; lisinopril; metformin; metoprolol; Symbicort; zifirlukast; carvedilol; clonidine; nifedipine; spironolactone; atorvastatin; baclofen; Colcrys; lactulose; omeprazole; insulin glargine; salbutamol; tramadol; dulaglutide; azilsartan; Twinrix	Recovered, resolved. **Possible**
TEN-01-301-(b) (6)	41WM	T	1.1 to 2.2	2.2 to 3.0	0.5	>30	V6 D1	Clonazepam; Vit B; acetaminophen/aspirin; venlafaxine; fiber; sertraline	Not resolved, still elevated at V8 but improving on study drug **Unlikely**

Patient and Study Number	Demo-graphics	Arm	Shift ALT (X ULN)	Shift AST (X ULN)	Max Post-baseline TBili (X ULN)	BMI	Visit (V) and Day (D)	Concomitant Meds	Outcome and Assessment of DILI Likelihood
TEN-01-302-(b) (6)	36HM	T	1.7 to 2.8	1.3 to 2.6	0.7	>30	V6 D93	PEG 3350; Theraflu; diphenhydramine with paracetamol; phenylephrine	Back to baseline at V9/ET **Unlikely**
TEN-01-302-(b) (6)	51HF	T	1.3 to 2.5	1.0 to 1.5	0.3	>30	V6 D91	Mometasone furoate; folic acid; etanercept; methotrexate; sulfasalazine; gabapentin; tizanidine; macrogol 3350; omeprazole	Improving at last visit **Possible**
TEN-01-302-(b) (6)	46WM	T	1.5 to 2.9	1.6 to 2.6	0.8	<30	V6 D85	Carvedilol	Improving ,not resolved **Possible**
TEN-01-302-(b) (6)	51 WF	P	1.5 to 2.1	1.6 to 2.0	1.5 (baseline) 1.4 (post-treatment)	<30	V4 D31	Relpax; Miralax; Crestor; indomethacin	Not resolved but not worse at ET **Unlikely**

* The only case that represents l kely DILI
Abbreviations: W, white; H, Hispanic; F, female; M, male; T, tenapanor; P, placebo; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DILI, drug-induced liver injury; MVI, multivitamin infusion; ULN, upper limit of normal; ET, end of treatment; PEG, polyethylene glycol
Source: Reviewer's table, derived from Applicant's Lb STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

In summary, within the controlled clinical trials, no difference was noted in proportion of patients who had elevation of ALT on treatment compared to placebo. The additional analyses of the Integrated Safety Analysis Set identified a small number of patients who experienced a rise in one or more liver enzymes, but given limitations of the integrated analysis, this was not considered a clear signal of drug induced liver injury. The rises were generally small, not associated with serious adverse events or poor outcomes, and none met Hy's law criteria. Thus, these data support inclusion of the terms "increase in AST and ALT" within the product label as a less common AE.

Potassium

Hyperkalemia was examined in the IBS-C population because of increased rates of hyperkalemia AEs noted in the ESRD and CKD Safety Sets. Hyperkalemia also occurred in some knock-out animals fed a low-sodium diet. This finding is likely due to an intracellular mechanism to compensate for excess export of sodium. Therefore, shift tables were created for hyperkalemia by Common Terminology Criteria for Adverse Events (CTCAE) Grade comparing tenapanor to placebo patients in the two phase 3 trials (Table 45 and Table 46) and the Integrated Safety Analysis Set (Table 65, Clinical Appendices, Section 16.1.3).

Phase 3 Trials

Shifts in potassium were analyzed for each of the phase 3 trials separately. Only patients with post-randomization values higher than those obtained at baseline were included in the analysis. The randomized withdrawal period of Study 301 was excluded from the analysis to prevent confounding issues due to study design. As seen in Table 45 and Table 46, a slight increase in cumulative incidence of hyperkalemia was found in tenapanor-treated patients in Study 301 (7.8% versus 5.0%, 37 vs 22/100 PY), but a decrease in the rate of hyperkalemia was found in Study 302 (7.2% versus 11.3%, 17/100 vs 26/100 PY). In both studies, a slight increase in the rate of hyperkalemia was noted when a higher threshold (>5.5 vs 5.0) was utilized. The reasons for these divergent results are unclear, but given that the findings were not confirmed in both studies, the majority of reported cases were minimally elevated (in the >5.0 and ≤5.5 range) and lack of imbalance in AEs reported related to hyperkalemia, a clear signal of hyperkalemia in IBS-C patients was not identified. The signal was also separately evaluated in patients with renal impairment (refer to section 10.2.11 below) and no increased propensity to hyperkalemia was identified in IBS-C patients with renal impairment.

Table 45. Abnormal Total Potassium Shifts From Baseline, Study 301, First 12 Weeks

	Treatment Arm		
	Tenapanor (N=309)	**Placebo (N=301)**	
Degree of Shift and Baseline Level	*64 PY*	*67 PY*	**CTCAE Grade**
Highest postrandomization value (normal at beginning of study)			
K >7 (~1.4 ULN)	0 (0%)	1 (0.33%)	4
K >6 (~1.2 ULN), ≤7	2 (0.65%)	1 (0.33%)	3
K >5.5 (~1.1 ULN), ≤6	4 (1.3%)	0 (0%)	2
K>5.0, ≤5.5	15 (4.5%)	8 (3.0%)	1
Highest postrandomization value (high at beginning of study)			
K >7	0 (%)	0	4
K >6, ≤7	1 (0.32%)	0 (0%)	3
K >5.5, ≤6	1 (0.32%)	0 (0%)	2
K >5.0, ≤5.5	1 (0.32%)	5 (1.7%)	1
Total K elevation (>5.5), all patients	8 (2.7%)	2 (0.66%)	All
	12.5/100 PY	*3.0/100 PY*	
Total K elevation (>5), all patients	24 (7.8%)	15 (5.0%)	All
	37/100 PY	*22/100 PY*	

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; K, potassium; ULN, upper limit of normal
Source: Reviewer's table, derived from Applicant's LB STDM data set, submitted September 12, 2018, analyzed with JMP 13.0 software

Table 46. Abnormal Total Potassium Shifts From Baseline, Study 302

	Treatment Arm		
	Tenapanor (N=293)	**Placebo (N=300)**	
Degree of Shift and Baseline Level	*123 PY*	*132 PY*	**CTCAE Grade**
Highest postrandomization value (normal at beginning of study)			
K >7 (~1.4 ULN)	1 (0.34%)	0 (0%)	4
K >6 (~1.2 ULN), ≤7	1 (0.34%)	1 (0.33%)	3
K >5.5 (~1.1 ULN), ≤6	4 (1.4%)	5 (1.7%)	2
K>5.0, ≤5.5	13 (5.8%)	27 (10%)	1
Highest postrandomization value (high at beginning of study)			
K >7	0 (0%)	0 (0%)	4
K >6, ≤7	0 (0%)	0 (0%)	3
K >5.5, ≤6	0 (0.34%)	0 (0%)	2
K >5.0, ≤5.5	2 (3.1%)	1 (0%)	1
Total K elevation (>5.5)	6 (2.4%)	6 (2.0%)	All
	4.9/100 PY	*4.5/100 PY*	
Total K elevation (>5)	21 (7.2%)	34 (11.3%)	All
	17/100 PY	*26/100 PY*	

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; K, potassium; ULN, upper limit of normal
Source: Reviewer's table, derived from Applicant's LB STDM data set, submitted September 12, 2018, analyzed with JMP 13.0 software

An Information Request was sent to the Applicant on March 28, 2019 requesting confirmation of our findings regarding hyperkalemia. The Applicant replied on April 12, 2019. Although the Applicant's findings were similar to those listed here, the magnitude of difference in hyperkalemia rates between tenapanor- and placebo-treated patients for Study 301, first 12 Weeks (see Table 45) was smaller according to the Applicant's analyses. The Applicant counted

laboratory tests obtained at the first study visit for patients rerandomized in the withdrawal phase as belonging to patients in the first 12 weeks of the study. We elected not to combine data from rerandomized patients with data from the first 12 weeks. Some of these patients, who received placebo in the first 12 weeks, had received at least one dose of study drug before their laboratory assessment at the first randomized withdrawal visit.

In the Applicant's response to the March 28, 2019 Information Request, the Applicant opined that the differences (in opposite directions) between tenapanor and placebo patients seen in the individual phase 3 studies could be due to chance, or an artifact secondary to difficult blood draws.

Similar findings were noted in the integrated safety analysis set, details of which appear in Clinical Appendix 16.1.3.

Other populations:

Of note, the signal of hyperkalemia was more pronounced in a population of CKD patients (see section 10.2.12) and rare findings of serious hyperkalemia that occurred in this population are noted in the label.

10.2.8. Adverse Events of Special Interest

The Applicant identified three adverse events of special interest in their Integrated Summary of Safety based on the known mechanism of action of the drug: diarrhea, dehydration and hyponatremia. As reviewed above, diarrhea was a common and significant AE across all studies. No signal of hyponatremia was identified. Regarding dehydration, reported AEs of dehydration were uncommon, occurring in 2 patients on tenapanor and 0 patients on placebo across the 2 confirmatory trials. Laboratory shift analyses for creatinine and BUN showed small increases from baseline associated with tenapanor.[5] However, the difference between the 2 arms was small, and, in isolation, without AEs reported, does not appear to represent a strong signal of dehydration associated with treatment.

The review team identified two additional areas of interest in the IBS-C safety dataset, based on the known mechanism of action of the drug, postmarketing data obtained from drugs with similar mechanisms of action (linaclotide), and results from ESRD and CKD datasets. These are: colitis, including inflammatory bowel disease, and hyperkalemia. Hyperkalemia has been discussed under Laboratory Findings in Section 10.2.7. Colitis will be discussed below. In addition, brief mention will be made of cardiovascular AEs due to finding of possible increased thrombosis in the ESRD data set (See Section 10.2.12).

[5] **Study 301**: 18/309 (5.8%) of tenapanor patients vs 11/301 (3.7%) of placebo had post-treatment creatinine > ULN
23/309 (7.4%) of tenapanor patients vs 22/301 (7.3%) of placebo patients had BUN > ULN
Study 302: 39/293 (13.3%) of tenapanor patients vs 34/300 (11.3%) of placebo patients had post-treatment creatinne > ULN
54/293 (18.4%) of tenapanor patients vs 49/300 (16.3%) of placebo patients had post-treatment BUN > ULN

Colitis

Drug-induced colitis, including inflammatory bowel disease, is a concern for patients on tenapanor based on:

- Reports showing the development of spontaneous colitis in mice with targeted disruption of the NHE3 gene
- Depletion of goblet cells and mucosal inflammation and bleeding on histologic examination of colonic tissue from rodents in the context of diarrhea in the preclinical program
- Development of chronic diarrhea and spontaneous, mild, inflammatory bowel disease in patients with inactivating mutations in the NHE3 gene

Two reports in the peer-reviewed literature express concern about the potential of small molecule inhibitors of NHE3 to trigger colitis or inflammatory bowel disease (Gurney et al. 2017; Rieg and Dominguez Rieg 2017).

Review of TEAEs in the Integrated Safety Analysis Set and in the two individual phase 3 trials revealed increased rates of rectal bleeding in tenapanor-treated patients compared to placebo (although absolute numbers are small). While there are other causes of rectal bleeding (for example, hemorrhoids and polyps), rectal bleeding/hematochezia is an AE that could be used to screen for colitis. Because the protocol did not require or recommend colonoscopy to evaluate new onset cases of rectal bleeding, it is not possible to definitely exclude or identify potential cases of colitis, including inflammatory bowel disease.

Rectal bleeding occurred more frequently in tenapanor-treated patients than placebo-treated patients. There were seven cases (7/1343, 0.52%, 1.5 per 100 person-years) of new-onset of rectal bleeding in patients on tenapanor compared to one case of new-onset of rectal bleeding in patients on placebo (1/738, 0.13%, 0.004 per person-year; Table 47 below). In Study 301, 4/309 (1.3%) patients on tenapanor and 0/301 (0%) patients on placebo had rectal bleeding. In Study 302, 3/293 (1.0%) patients on tenapanor and 1/300 (0.33%) on placebo had rectal bleeding.

None of the patients with rectal bleeding had significant changes in laboratory parameters or vital signs except the placebo patient who developed acute anemia during a rectal hemorrhage. In all patients, the rectal bleeding reportedly resolved by the end of the study.

Only two of the patients with rectal bleeding underwent colonoscopy; one was a 61-year-old, female, placebo patient (TEN-01-302, (b) (6)) with rectal hemorrhage who was found to have a polyp, which was removed. The second case, a 58-year-old woman who had microscopic heme-positive stool, was a tenapanor-treated patient (TEN-01-302, (b) (6)). On colonoscopy, this patient was found to have a small polyp and internal hemorrhoids but no evidence of colitis.

We identified at least three cases whose narratives were consistent with possible colitis, for whom sufficient information to ascribe the rectal bleeding to another cause was not collected or was not available.

1) TEN-01-301, (b) (6), was a 45-year-old woman with a previous history of ischemic colitis, who had hemorrhagic diarrhea but did not undergo colonoscopy or imaging. Therefore, it is unknown whether her symptoms constituted a recurrence of ischemic colitis. At the end of the study, the rectal bleeding had resolved but the patient's diarrhea persisted requiring follow-up with a gastroenterologist. The details of this follow-up are not available. Given the short onset to PD effect and short half-life, the fact that the diarrhea did not quickly resolve upon drug discontinuation was concerning for possible low grade colitis.

2) TEN-01-301, (b) (6) was a 42-year-old woman with two episodes of rectal bleeding on tenapanor, who ultimately withdrew from the study due to complaints of proctalgia and diarrhea. Follow-up information is not available.

3) TEN-01-301, (b) (6) was a 56-year-old woman on tenapanor who experienced rectal bleeding. The rectal bleeding was reported to have resolved at the end of the randomized treatment period. However, 18 days after enrolling in the extension study, Study303, the patient developed hemorrhagic diarrhea on tenapanor. There is no narrative available for this patient and, therefore, other causes of hemorrhagic diarrhea cannot be excluded, and drug-induced colitis remains a possibility.

The following table (Table 47) provides a summary of the 7 cases of rectal bleeding that occurred on tenapanor.

Table 47. Rectal Bleeding AEs in Tenapanor-treated Patients by Study, Subject ID, Demographics, Treatment Days, Outcome and Other AEs

Study, Subject ID	Demographics	Study Days of AE	Other AEs (Days)	Outcome
TEN-01-301 (b) (6)	42BF	64-71	Diarrhea (1, 85-89); Proctalgia (85-89)	Resolved. No colonoscopy
TEN-01-301 (b) (6)	62WF	56-83	Diarrhea (88-ET)	Bleeding resolved. Diarrhea not resolved (saw GI); history of ischemic colitis and C. difficile in past. No colonoscopy.
TEN-01-301 (b) (6)	22BF	31-33	Diarrhea (31-48)	Resolved. No colonoscopy.
TEN-01-301 (b) (6)	56WF	21(RW)	Bloody diarrhea (18-21/TEN-01-303)	No narrative available. "Resolved" but recurred in extension study. No colonoscopy.
TEN-01-302 (b) (6)	45WF	17-18		Resolved. Had negative CT. No colonoscopy
TEN-01-302 (b) (6)	58BF	17-ET		Heme-positive stool. No overt blood. Colonoscopy: Polyps, internal hemorrhoids
TEN-01-302 (b) (6)	28WF	2-6	ADHD (7-44)	Resolved.

Abbreviations: AE, adverse event; CT, computed tomography; ET, end of treatment; RW, randomized-withdrawal; W, white; B, black; F, female
Source: Reviewer's table, derived from Applicant's AE data listings for studies, D5612C0001, RDX5791-201, TEN-01-301, TEN-01-302, submitted September 12, 2018

Based on the information summarized above, there is insufficient evidence to determine if tenapanor-associated new-onset colitis, including inflammatory bowel disease, has occurred. If colitis occurred in this study population, it is likely to have been mild, given that no alterations in laboratory data or vital signs occurred in most of the patients with rectal bleeding. However, the possibility exists that inflammatory bowel disease could be triggered in genetically susceptible individuals.

Whereas the numbers of patients experiencing rectal bleeding were low, rectal bleeding may represent a "tip of the iceberg" phenomenon in patients with mild colitis. The clinical symptoms of animals who developed histologic evidence of colitis and depletion of goblet cells in the preclinical program included, for the most part, diarrhea, and not rectal bleeding. Given that diarrhea is a common, expected adverse event associated with drugs used to treat constipation, and the protocol did not mandate colonoscopy or other testing to evaluate new onset diarrhea before ascribing it to pharmacodynamic effect of the drug, uncertainty remains. In clinical practice, patients with mild colitis, particularly right-sided colitis, often present with diarrhea in the absence of rectal bleeding. Thus, colonoscopy with biopsy evaluated for histologic changes would be the only way to determine with accuracy whether mild mucosal inflammation is occurring. It is possible that rectal bleeding, and the attendant risk of colitis, is a class effect shared by all secretagogues that inhibit NHE3 (including guanylate cyclase agonists which act

upstream of tenapanor in a shared pathway); insufficient data are available to confirm or refute this risk. While the data available in tenapanor treated patients with rectal bleeding are limited and do not confirm a safety signal that would preclude approval (given small absolute number of patients, lack of documented serious outcomes associated, and uncertainty regarding underlying cause of the bleeding in the majority of the documented cases), we intend to further evaluate the potential of tenapanor to induce colitis in the post-marketing setting, by conducting a signal assessment study in Sentinel. (Refer to Section 10.2.13 below.)

Cardiovascular AEs

(b) (4), a possible imbalance in the number of ischemic-thromboembolic AEs in patients receiving tenapanor for hyperphosphatemia and ESRD was noted (see Section 10.2.12 below). As a result, a detailed analysis of all cardiovascular AEs in the Integrated Safety Analysis Set for IBS-C patients was conducted. A consultation was requested from the Division of Cardiovascular and Renal Products (DCaRP) to assist in this evaluation. The DCaRP consultant recommended analyzing cardiovascular events in groupings based on pathophysiology, and evaluating stent/shunt-related thrombotic events separately in the ESRD population. Based on re-analysis of the IBS-C cardiovascular AEs informed by discussions with DCaRP, no clear increase in cardiovascular AEs was noted (Table 48. Cardiovascular AEs, Integrated Safety Analysis Set, by Category). Refer to consult by Dr. Stephen Grant (DCaRP) for additional detail.

Table 48. Cardiovascular AEs, Integrated Safety Analysis Set, by Category

Diagnosis	Tenapanor N=1343 466.1 PY	Placebo N=438 222 PY
Total CV deaths, MI and stroke	0/1343 (0%) *0/100 PY*	0/438 (0%) *0/100 PY*
Total congestive heart failure requiring hospitalization	0/1343 (0%) *0/100 PY*	0/438 (0%) *0/100 PY*
Total atrial arrhythmias	2/1343 (0.15%) *0.4/100 PY*	0/438 (0%) *0/100 PY*
Total miscellaneous serious CV events	1/1343 (0.07%) *0.2/100 PY*	0/438 0% *0/100 PY*
Total venous thromboembolisms	1/1343 (0.07%) *36/100 PY*	2/438 (0.45%) *90/100 PY*
Total significant cardiovascular AEs	4/1343 (0.30%) *85/100 PY*	2/438 (0.45%) *90/100 PY*

Abbreviations: AE, adverse event; CV, cardiovascular; MI, myocardial infarction; PY, person-years
Source: Reviewer's table, derived from Applicant's AE ADAM data sets for studies D5612C001, TEN-01-301, TEN-01-302, TEN-01-303, submitted September 12, 2018, analyzed with JMP v. 13

10.2.9. Other Safety Evaluation (Vital Signs, Cardiovascular safety)

Vital signs

Analysis of vital signs was performed for the Integrated Safety Analysis Set. No significant differences were detected in mean heart rate, blood pressure, or temperature over time between placebo and tenapanor or across dosage groups for tenapanor.

Electrocardiograms

Analyses of ECG results (PR interval, QRS interval and QT interval) were conducted for the Integrated Safety Analysis Set. No significant differences or trends were noted for patients treated with tenapanor as compared to placebo and among dosage groups for tenapanor. Further analyses were conducted comparing proportion of patients in study TEN-01-301 and TEN-01-302 whose ECG readings shifted from normal at baseline to abnormal (not clinically significant) at end of treatment visits. No significant differences between placebo and tenapanor groups were detected. No patients developed clinically significant ECG changes during either of the two trials.

QT

No difference in mean QT intervals was noted in the ECG analysis. No difference was found between placebo- and tenapanor-treated patients in the incidence of prolonged QT interval in the Integrated Safety Analysis data set. The Division agreed at the EOP2 meeting that a thorough QT study was not needed. See Section 3.2 on Regulatory Background. A consultation from the QT Interdisciplinary Studies Review Team was finalized on January 28, 2019. The consult concluded that “no significant QT prolongation by tenapanor was detected in this assessment.”

10.2.10. Safety Analyses by Demographic Subgroups

Analyses of all TEAEs, as well as three AEs of interest, diarrhea, rectal bleeding, and hyperkalemia, were conducted by age, sex, and race.

Analysis of AEs by Demographic Subgroup (Trial 301)

Rates of common, serious, and severe intensity adverse events were assessed by demographic subgroups for each of the phase 3 trials.

For trial 301 (Table 49 and Table 50), no substantial difference was noted in the risk difference in AEs based on age. Women on tenapanor seemed to have higher risk differences vs placebo compared to men on tenapanor for severe AEs (6.9 vs 3.1%), TEAEs (34 vs 16%) and diarrhea (24 vs 12%). However, the width of the confidence intervals around the estimates and lack of replication of the female-male drug-placebo differences in trial 302, suggest significant uncertainty about the female-males risk differences. Whites on tenapanor appeared to have

higher risk differences vs placebo compared to blacks on tenapanor vs placebo for diarrhea (28 vs 19%) but the notable overlap in the confidence intervals of the estimates suggest significant uncertainty about a risk differences. Note that, in general, there is considerable uncertainty around these subgroup comparisons (as evident in the wide confidence intervals around the estimated risk differences) due to small sample sizes in several subgroups and the multiple comparisons being made.

Table 49. Percentage of Patients with SAEs and Severe AEs by Treatment Arm and Demographic Subgroup (Study 301)

No. of Patients	Safety Population on Tena.	Safety Population on Placebo	SAEs on Tena.	SAEs on Placebo	SAE Risk Difference (%, 95% CI)	Severe AEs on Tena.	Severe AEs on Placebo	Severe AE Risk Difference (%, 95% CI)
Sex								
Male	64	50	1 (1.5%)	0 (0%)	**1.5 [-1.5,4.5]**	2 (3.1%)	0 (0%)	**3.1 [-0.9,7.1]**
Female	245	251	6 (2.4%)	0 (0%)	**2.4 [0.4,4.4]**	18 (7.3%)	4 (0.41%)	**6.9 [3.9,9.9]**
Age								
≤65	284	281	7 (2.5%)	0 (0%)	**2.5 [0.5,4.5]**	19 (6.7%)	4 (1.4%)	**5.3 [2.3,8.3]**
>65	25	20	0 (0%)	0 (0%)	**0**	1 (4%)	0 (0%)	**4 [-4,12]**
Race								
White	202	188	6 (3.0%)	0 (0%)	**3 [1,5]**	16 (7.9%)	4 (2.1%)	**5.8 [1.8,9.8]**
Black	89	100	0 (0%)	0 (0%)	**0**	4 (4.5%)	0	**4.5 [0.5,8.5]**
Asian	10	4	0 (0%)	0 (0%)	**0**	0(0%)	0	**0**
Native American, Hawaiian	0	2	0 (0%)	0 (0%)	**0**	0(0%)	0	**0**
Other, multiple, unknown	8	7	1 (12.5%)	0 (0%)	**12.5 [-10.5,35.5]**	0(0%)	0	**0**

Abbreviations: AE, adverse event; SAE, serious adverse event; Tena., tenapanor; TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from Applicant's ADSL and AE data files for Study,TEN-01-301, submitted September 12, 2018

Table 50. Percentage of Patients with Any TEAEs and Diarrhea by Treatment Arm and Demographic Subgroup (Study 301)

No. of Patients	Safety Population on Tena.	Safety Population on Placebo	TEAEs on Tena.	TEAEs on Placebo	TEAE Risk Difference (%, 95% CI))	Diarrhea on Tena.	Diarrhea on Placebo	Diarrhea Risk Difference (%, 95% CI)
Sex								
Male	64	50	24 (38%)	11 (22%)	**16 [-1,33]**	8 (12%)	0 (0%)	**12 [4,20]**
Female	245	251	164 (67%)	83 (33%)	**34 [26,42]**	72 (29%)	5 (2%)	**27 [21,31]**
Age								
≤65	284	281	175 (62%)	90 (32%)	**30 [22,38]**	74 (26%)	5 (1.8%)	**24 [19,29]**
>65	25	20	13 (52%)	4 (20%)	**32 [6,58]**	6 (24%)	0 (0%)	**24 [7,41]**
Race								
White	202	188	125 (62%)	54 (29%)	**33 [24,42]**	56 (29%)	2 (1.1%)	**28 [22,34]**
Black	89	100	53 (60%)	34 (34%)	**26 [12,40]**	19 (21%)	2 (2%)	**19 [10,28]**
Asian	10	4	5 (50%)	2 (50%)	**0**	1 (10%)	1 (25%)	**-15 [-61,31]**
Native American or Hawaiian	0	2	0 (0%)	0 (0%)	**0**	0 (0%)	0 (0%)	**0**
Other, multiple, unknown	8	7	5 (62%)	4 (57%)	**5 [-45,55]**	0 (0%)	0 (0%)	**0**

Abbreviations: AE, adverse event; SAE, serious adverse event; Tena., tenapanor; TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from Applicant's ADSL and AE data files for Study,TEN-01-301, submitted September 12, 2018

Analysis of AEs by Demographic Subgroup (Trial 302)

For trial 302 (Table 51 and Table 52), the small number of geriatric patients included (< 25 per group) and the considerable imbalance in the number of patients exposed to tenapanor by age group (> 10:1 ratio) preclude an informative comparison by age. Whites on tenapanor seemed to have a higher risk differences vs placebo as compared to blacks on tenapanor for TEAEs (10 vs 7%, Table 52) and diarrhea (17 vs 5.4%, Table 52); however, the overlap of the confidence intervals around the estimates again results in uncertainty regarding the true risk difference. Further, the risk difference for Severe AEs was actually less in whites than in blacks (0.7% vs 2.1%).

Table 51. Percentage of Patients with SAEs and Severe AEs by Treatment Arm and Demographic subgroup (Study 302)

No. of Patients	Safety Population on Tena. N=293	Safety Population on Placebo N=300	SAEs on Tena.	SAEs on Placebo	SAEs Risk Difference (%, 95% CI)	Severe AEs on Tena.	Severe AEs on Placebo	Severe AEs Risk Difference (%, 95% CI)
Sex								
Male	53	53	2 (3.8%)	0 (0%)	**3.8 [-1.2,8.8]**	1 (1.9%)	0 (0%)	**1.9 [-2.1,5.9]**
Female	240	247	2 (0.83%)	7 (2.8%)	**-2.0 [-4,0]**	11 (4.6%)	9 (3.6%)	**1 [-3,5]**
Age								
≤65	273	276	4 (1.5%)	6 (2.2%)	**-0.7 [-2.7,2.0]**	12 (4.4%)	9 (3.3%)	**1.1 [-1.9,4.1]**
>65	20	24	0 (0%)	1 (4.2%)	**-4.2 [-12,12]**	0 (0%)	0 (0%)	**0**
Race								
White	185	192	4 (2.2%)	8 (4.2%)	**-2 [-6,2]**	8 (4.3%)	7 (3.6%)	**0.7 [-3.3,4.7]**
Black	92	92	0 (0%)	3 (3.3%)	**-3.3 [-7.3,1.3]**	4 (4.3%)	2 (2.2%)	**2.1 [-2.9,7.1]**
Asian	12	9	0 (0%)	0 (0%)	**0**	0 (0%)	0 (0%)	**0**
Native American or Hawaiian	1	0	0 (0%)	0 (0%)	**0**	0 (0%)	0 (0%)	**0**
Other, multiple, unknown	3	7	0 (0%)	0 (0%)	**0**	0 (0%)	0 (0%)	**0**

Abbreviations: AE, adverse event; SAE, serious adverse event; Tena., tenapanor; TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from Applicant's ADSL and AE data files for Study,TEN-01-302, submitted September 12, 2018

Table 52. Percentages of Patients with Any TEAEs and Diarrhea by Treatment Arm and Demographic Subgroup (Study 302)

No. of Patients	Safety Population on Tena. N=293	Safety Population on Placebo N=300	TEAEs on Tena. N=146	TEAEs on Placebo	TEAEs Risk Difference (%, 95% CI)	Diarrhea on Tena. N=48	Diarrhea on Placebo	Diarrhea Risk Difference (%, 95% CI)
Sex								
Male	53	53	22 (42%)	17 (32%)	**10 [-8,28]**	7 (13%)	0 (0%)	**13 [8,18]**
Female	240	247	124 (52%)	108 (44%)	**8 [-1,17]**	41 (17%)	11 (4.5%)	**12.5 [7.5,17]**
Age								
≤65	273	276	136 (50%)	115 (42%)	**8 [0,16]**	46 (17%)	11 (4.0%)	**13 [8,18]**
>65	20	24	10 (50%)	10 (42%)	**8 [-21,37]**	2 (10%)	0 (0%)	**10 [-3,23]**
Race								
White	185	192	92 (50%)	77 (40%)	**10 [0,20]**	39 (21%)	8 (4.2%)	**17 [10,24]**
Black	92	92	49 (53%)	42 (46%)	**7 [-7,21]**	8 (8.7%)	3 (3.3)	**5.4 [-1.6,12]**
Asian	12	9	3 (25%)	4 (44%)	**-19 [-60,22]**	0 (0%)	0 (0%)	**0**
Native American or Hawaiian	1	0	0 (0%)	0 (0%)	**0**	0 (0%)	0 (0%)	**0**
Other, multiple, unknown	3	7	2 (67%)	2 (29%)	**38 [-25,101]**	1 (33%)	0 (0%)	**33 [-20,86]**

Abbreviations: AE, adverse event; SAE, serious adverse event; Tena., tenapanor; TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from Applicant's ADSL and AE data files for Study,TEN-01-302, submitted September 12, 2018

Supportive Safety Analyses

As shown in Table 66 and Table 67 (Clinical Appendix, Section 16.1), in the Integrated Safety set, no clear pattern was noted in the risk difference in SAEs, severe AEs, TEAEs, or diarrhea between tenapanor and placebo based on sex, age, or race when the confidence intervals around the estimates are taken into account and incidence rates are compared.

10.2.11. Safety Analysis in Renally Impaired Patients

Based upon findings identified during exploration of the ESRD and CKD supportive safety sets (discussed in 10.2.12 below), the safety profile of tenapanor in IBS-C patients with concomitant renal impairment was explored.

Within the IBS-C trial program, the applicant enrolled an adequate proportion of patients with renal impairment. Renal impairment was defined as eGFR <90ml/min/1.73m^2 (estimated based

on CKD-EPI equation). Three hundred sixty-eight patients with renal impairment were enrolled in the phase 3 program, representing 31% of the overall population.

In response to IR provided on 7/19/19, the sponsor summarized laboratory data (shift from baseline in potassium) by baseline renal function and treatment group. In (Table 53) below the data show that overall, tenapanor-treated, IBS-C patients with renal impairment experienced fewer elevations in potassium than those treated with placebo. There were no reported AEs of hyperkalemia in IBS-C patients with eGFR <90ml/min/1.73m^2.

Table 53. Proportion of Patients with Elevated Post-Dose Potassium (>5mmol/L) by baseline Renal Function (Controlled period of Studies 301 and 302 combined)

	Impaired Renal Function (eGFR < 90)		Normal Renal Function (eGFR ≥ 90)	
	IBSRELA	Placebo	IBSRELA	Placebo
All	15/194 (7.7%)	16/174 (9.2%)	35/407 (8.6%)	42/426 (9.9%)
Subgroup				
Max Pre-Dose K ≤ 5	12/184 (6.5%)	13/169 (7.7%)	31/396 (7.8%)	40/408 (9.8%)
Max Pre-Dose K > 5	3/10 (30.0%)	3/5 (60.0%)	4/9 (44.4%)	2/17 (11.8%)

* Two IBSRELA-treated patients ([(b) (6)] from Study 1 and [(b) (6)] from Study 2) and one placebo-treated patient ([(b) (6)] from Study 2) had creatinine data at baseline but did not have any pre-dose potassium data. They are excluded from the subgroup analyses by maximum pre-dose potassium level.

Source: sponsor's response to IR dated 7/19/19. Analysis of double-blind periods of study 301 and 302.

Additionally, the sponsor provided analysis of the proportion of patients experiencing any TEAE, SAE, Diarrhea, Severe Diarrhea, Hyperkalemia reported as an AE, and a pooled analysis of events of interest (including changes in electrolytes, dehydration, fall) which could be interpreted as consequences of diarrhea and/or resultant dehydration. This analysis identified that patients with renal impairment appeared to have more frequent diarrhea and severe diarrhea than IBS-C patients with normal renal function. However, reassuringly, patients with renal impairment did not appear to suffer an increased risk of complications from diarrhea.

In the following table (Table 54), the results of these analyses are summarized. The label will note that patients with renal impairment may experience more frequent or severe diarrhea.

Table 54. Rates of TEAEs, SAEs, AEs of Diarrhea, Hyperkalemia, and Events of Special Interest by Baseline Renal function (First 12-Week Treatment Period, Studies 301 and 302 Combined)

	Impaired Renal Function (eGFR < 90)		Normal Renal Function (eGFR ≥ 90)	
Subjects with Any	**IBSRELA (N=194)**	**Placebo (N=174)**	**IBSRELA (N=407)**	**Placebo (N=426)**
TEAEs	92 (47.4%)	67 (38.5%)	161 (39.6%)	131 (30.8%)
SAEs	4 (2.1%)	3 (1.7%)	4 (1.0%)	3 (0.7%)
Diarrhea	39 (20.1%)	1 (0.6%)	53 (13.0%)	15 (3.5%)
Severe Diarrhea	8 (4.1%)	1 (0.6%)	7 (1.7%)	0
Hyperkalemia[§]	0	0	3 (0.7%)	2 (0.5%)
ESIs[†]	2 (1.0%)	6 (3.4%)	10 (2.5%)	7 (1.6%)
Blood Bicarbonate Decreased	1 (0.5%)	0	2 (0.5%)	0
Blood Calcium Increased	0	1 (0.6%)	1 (0.2%)	0
Blood Phosphorus Decreased	1 (0.5%)	0	0	1 (0.2%)
Blood Phosphorus Increased	0	0	1 (0.2%)	0
Blood Potassium Decreased	1 (0.5%)	0	0	0
Blood Potassium Increased	0	0	1 (0.2%)	0
Dehydration	0	s	2 (0.5%)	0
Fall	0	3 (1.7%)	1 (0.2%)	2 (0.5%)
Hyperkalaemia	0	0	2 (0.5%)	2 (0.5%)
Hypochloraemia	0	1 (0.6%)	0	0
Hypokalaemia	0	0	0	1 (0.2%)
Hyponatraemia	0	1 (0.6%)	1 (0.2%)	2 (0.5%)
Urine Calcium Increased	0	1 (0.6%)	0	0

[§] Reported TEAEs with the preferred term of *Hyperkalaemia* or *Blood Potassium Increased*

[†] Events of special interest: TEAEs related to dehydration and electrolyte abnormality, hypotension, syncope, and falls.

Source: Applicant's table, derived from Applicant's Table 1.1 in Clinical Response to Information Request, submitted July 19, 2019

10.2.12. Specific Safety Studies/Clinical Trials in Special Populations

ESRD Safety Data Analysis Set

Patients with renal disease have been treated with tenapanor for hyperphosphatemia under IND [redacted] (b) (4) in studies D5611C001, D5613C001 and TEN-02-201. Data from these studies, conducted under this open IND, provide safety information on the special population of patients with renal impairment.

Two deaths occurred in the ESRD population; one was due to left lower extremity ischemia resulting in cardiac failure and the other was due to intestinal obstruction (See Section 17.4.1 for further details).

Treatment-emergent SAEs for patients on tenapanor in the ESRD Safety Analysis Set occurred at similar rates to those seen in patients on placebo. In total, 57 SAEs occurred in 31/398 (7.8%) patients on tenapanor and 14 SAEs were reported in 11/151 (7.3%) placebo patients. There was no clear dose-response or SOC pattern for SAEs within this safety set.

In the ESRD Safety Set, tenapanor-treated patients (80/398, 20%) had higher rates of discontinuation due to AEs than placebo-treated patients (7/151, 4.6%). Of 80 AEs on tenapanor resulting in discontinuation, 52 (65%) were gastrointestinal and, of these, 37 were diarrhea (the most common AE resulting in discontinuation).

Severe adverse events occurred more frequently in tenapanor-treated ESRD patients (60/398,15%) than in placebo-treated ESRD patients (10/151,6.6%). The most common severe AEs were gastrointestinal, occurring in 44/97 (45%) of tenapanor-treated patients compared to 1/17 (5.9%) of patients on placebo. Additionally, nine patients had severe AEs in the infection and infestations SOC; eight were on tenapanor and one on placebo. Two patients on tenapanor had severe AEs of hyperkalemia compared to none on placebo.

Common, treatment-emergent AEs are listed for the ESRD group in Table 55. As might be expected, diarrhea was the most common TEAE on tenapanor but the total cumulative incidence of diarrhea (42% of tenapanor-treated patients) was substantially higher than that seen in the IBS-C group (14% of tenapanor-treated patients). After diarrhea, ischemic-thromboembolic events, a category we created, were the next most common AEs (see discussion below). Hyperkalemia AEs occurred in 2.5% of patients on tenapanor compared to 0.66% on placebo (see discussion in 10.2). Interestingly, hyponatremia, an AE of special interest based on the drug's mechanism of action, was not frequently observed in patients on tenapanor. A slight increase in the percentage of tenapanor-treated patients with injuries (fractures or falls) was also noted, which could be due to chance. However, it is possible that patients who are dehydrated and/or hypovolemic from diarrhea might present a greater fall risk. Overall, the risk benefit may be different for ESRD than for IBS-C, due to differences in age and/or medical co-morbidities.

Table 55. Common TEAEs with Risk Difference >1% Between Tenapanor and Placebo: ESRD Safety Data Analysis Set

Diagnosis	Tenapanor (n=398)**	Placebo (n=151)*
Diarrhea	169 (42%)	10 (6.6%)
Ischemic/thromboembolic AEs	32 (8%)	2 (1.3%)
Nausea and/or vomiting	26 (6.5%)	7 (4.6%)
Abdominal pain	21 (5.3%)	3 (2.0%)
Hyperkalemia	10 (2.5%)	1 (0.66%)
Cellulitis	7 (1.8%)	0 (0%)
Chest pain	8 (2.0%)	0 (0%)
Injuries	8 (2.0%)	1 (0.66%)

* Placebo arm includes patients rerandomized to placebo from tenapanor in the randomized withdrawal period of study, TEN-02-201; (some of these patients had previously received tenapanor)
** Represents all patients who received at least one dose of tenapanor
Abbreviations: AE, adverse event; ESRD, end-stage renal disease; TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from the Applicant's ADAE ADAM files, from Studies D5611C001, D5613C001 and TEN-02-201, submitted September 12, 2018

Because of the discovery of an increase in cases of ischemic/thromboembolic events in patients with ESRD on tenapanor, members of the Division of Cardiorenal Products (DCARP) with expertise in Nephrology were consulted and replied to the consult on March 13, 2019 (see Consult in DARRTS by Dr. Shen Xiao). The consultant noted that analyses of common TEAEs in the ESRD Safety Data Analysis Set were based on pooling results from 3, phase 2, clinical trials with different duration, design and dosing regimens. As a result, the findings could be spurious. DCARP recommended examining venous thromboembolic events alone because the pathophysiology of arterial and venous thromboembolic events can differ. When the data were reanalyzed to include only venous thromboembolic events and the data from only two comparable trials were compared, the rates of thromboembolic events were 5/180 (2.8%) in tenapanor-treated patients and 1/69 (1.5%) in placebo patients. Given the small sample size, the precision around these estimates is poor. Additionally this patient population has high rates of thromboembolic events in general as a result of their comorbid conditions. This small numeric difference between arms was not considered a strong signal.

CKD Safety Analysis Data Set

(b) (4)

Although this cohort of patients is, in general, older with more comorbidity than the IBS-C cohort, it is, in general, less chronically ill than the ESRD cohort. Thus, it may represent a reasonable population from whom to glean information about expected adverse events in older, IBS-C patients with comorbidities.

In the CKD Safety Analysis Set, SAEs occurred with similar frequency in tenapanor patients as in placebo; 6/77 (7.8%) of patients on tenapanor and 4/77 (5.2%) of placebo-treated patients developed a total of seven and five SAEs, respectively. Two of the SAEs on tenapanor were hyperkalemia (data not shown).

In the CKD Set, 20/77 (26%) tenapanor patients withdrew from the study due to AEs compared to 3/77 (3.9%) of placebo patients. Sixteen of the tenapanor patients (21%) withdrew due to gastrointestinal AEs; of these, all but one withdrew due to diarrhea (data not shown).

Tenapanor-treated patients (9/77,12%) had more severe AEs than placebo-treated patients (5/77,6.5%) in the CKD Safety Set (data not shown). As expected, the most common severe AEs were gastrointestinal with 6/77 (7.8%) patients on tenapanor reporting severe, gastrointestinal AEs and none on placebo. In contrast to the ESRD Safety Set, there was no difference in thromboembolic events between the two groups. However, the total number of patients (144) in this cohort was relatively small.

A higher cumulative incidence of diarrhea as a TEAE was identified in the CKD Safety Analysis Set, as compared to ESRD or IBS-C (Table 56). A total of 51 AEs of diarrhea occurred in 77 patients (66%) in the tenapanor treatment arm as compared to five in 77 patients (6.5%) in the placebo arm. This high rate of diarrhea may be in part explained by the fact that the drug has a pharmacodynamic effect to increase stool output. While this may be therapeutic in constipation, patients with renal disease using the drug for other purposes, who may have normal baseline stool pattern, are likely to report more bothersome “diarrhea” when exposed to the drug, compared to IBS-C patients. Other less common abdominal AEs included abdominal distension, abdominal pain and nausea and vomiting.

As with the ESRD patients, hyperkalemia AEs were more prevalent in the tenapanor-treated group (9.1%) than in the placebo group (1.3%). The Applicant identified hyperkalemia as a potential risk in the CKD population. Two of the patients with hyperkalemia AEs had SAEs of hyperkalemia that required hospitalization and were accompanied by EKG changes. Although rates of hyperkalemia were not elevated in IBS-C patients with renal impairment based on a pooled analysis of the controlled portions of studies 301 and 302 (refer to 10.2.11 above), these 2 cases of SAEs of hyperkalemia in CKD patients were life threatening, and warrant inclusion in the label as (b) (4)

As seen in the ESRD safety population, chest pain was slightly more prevalent in the tenapanor group 3/77 (3.9%) than in the placebo group 1/77 (1.3%). However on further evaluation, all 3 cases of chest pain reported on treatment were evaluated as “non-cardiac” or “musculoskeletal” chest pain. The CKD Data Set was the only dataset in which the FDA-created AE of dehydration/acute kidney injury occurred more commonly in tenapanor-treated patients than in placebo. Although underlying etiology for these changes might differ across patients (worsening of underlying renal disease, concomitant medications, etc. may confound the findings), it is notable that hypovolemia/dehydration was another of the potential risks identified by the Applicant. This imbalance may suggest that CKD patients are possibly more susceptible to volume depletion and complications, should they develop diarrhea, an AE that was highly prevalent. Although patients with CKD being treated for albuminuria may differ from those with IBS-C, these data suggest that this dehydration and/or acute kidney injury may be a concern in older, sicker patients and this may be relevant to IBS-C patients with a degree of renal impairment. This signal prompted further assessment of such AEs in the IBS-C population

with impaired baseline renal function (results described in section 10.2.11), and reassuringly, in that population, a signal was not apparent.

Table 56. Common TEAEs With Risk Difference >1% Between Tenapanor and Placebo: CKD Safety Data Analysis Set

Diagnosis	Tenapanor (n=77)	Placebo (n=77)
Diarrhea	51 (66%)	5 (6.5%)
Acute kidney injury/dehydration*	10 (13%)	5 (6.5%)
Hyperkalemia	7 (9.1%)	1 (1.3%)
Nausea and/or vomiting	7 (9.1%)	2 (2.6%)
Abdominal pain	4 (5.2%)	0 (0%)
Abdominal distension	3 (3.9%)	0 (0%)
Chest pain	3 (3.9%)	1 (1.3%)

Abbreviations: CKD, chronic kidney disease; TEAE, treatment-emergent adverse event
*Includes AE terms: acute kidney injury, dehydration, blood urea nitrogen increased, blood creatinine increased, decreased glomerular filtration rate
Source: FDA's table, derived from Applicant's line listing of AEs for Study D5610C0001, submitted September 12, 2018

Human Carcinogenicity or Tumor Development

There was no evidence of carcinogenicity in a 2-year rat carcinogenicity study. Two patients in the IBS-C development program developed malignancies while on study drug; one had a pineal tumor and the other had a laryngeal neoplasm. Neither malignancy was considered related to the study drug.

Human Reproduction and Pregnancy

Information on the outcome of pregnancies initiated while on tenapanor was limited. Four patients became pregnant inadvertently during the IBS-C trials. Two patients enrolled in study TEN-01-302 were receiving placebo at the time of their pregnancy. Two patients who received tenapanor in study TEN-01-301 are described below.

The first patient, TEN-010-301/ (b) (6), received tenapanor for 12 weeks followed by placebo for 4 weeks. Her last menstrual period and her last dose of study drug were on (b) (6) She started placebo after (b) (6). She was found to be pregnant while on placebo on (b) (6), 1 month after stopping study drug. She was told to continue taking the study drug (it is unclear if the investigator who told her this was unblinded). The only available follow up information states that a prenatal test showed no evidence of birth defects.

The second patient, TEN-010-301/ (b) (6), received tenapanor for 4 weeks starting on (b) (6) and had unprotected sex on (b) (6). She asked the principal investigator for a Plan B prescription on (b) (6). She had a positive pregnancy test (human chorionic gonadotropin) on (b) (6) and repeat pregnancy test on (b) (6) showed declining values that the principal investigator interpreted as compatible with pregnancy loss.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

No evidence of drug abuse potential, withdrawal or rebound effect has been shown in any of the trials to date.

An ADME study in rats showed no evidence that tenapanor crosses the blood-brain barrier. There have been no reported cases of overdosage of tenapanor in humans during the clinical development period. Healthy volunteers have received doses as high as 900 mg of tenapanor with minimal systemic exposure. In animals, high dosages have resulted in diarrhea and dehydration and, in isolated cases, colonic bleeding.

Regarding the potential for rebound effects, adverse events were assessed in the randomized withdrawal period of study 301. The Applicant submitted data on the 18 AEs reported in patients receiving placebo in the randomized withdrawal arm of study 301, who had received tenapanor in the 12-week, double-blind period of the trial. No specific SOC was overrepresented. Only one AE, nausea, was gastrointestinal. Thus, the results of this trial do not support concerns about withdrawal or rebound effects manifesting as AEs within a specific SOC after stopping the drug.

10.2.13. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

Tenapanor is not marketed elsewhere in the world; therefore, no postmarketing safety data are available at this time.

Expectations on Safety in the Postmarket Setting

The drug's mechanism of action presents a theoretical safety concern for the development of IBD among those exposed to tenapanor. Given this theoretical concern, the Agency will conduct a postmarketing signal detection study to further assess whether postmarket data show a signal of IBD risk in patients treated with tenapanor using the Active Risk Identification and Analysis system in the Sentinel Distributed Database.

10.3. Integrated Assessment of Efficacy and Safety

The results of two randomized, placebo-controlled, double-blind, multicenter, phase 3 studies, 301 and 302, support efficacy of tenapanor in the treatment of IBS-C. The primary endpoint was the proportion of patients who were weekly overall responders at 6 or more of the first 12 weeks of double-blind treatment. An overall responder was defined as a weekly responder for at least 6/12 weeks where both CSBM (increase of one or more in average weekly CSBMs from baseline) and abdominal pain response (decrease of 30% or more in average, weekly, worst abdominal pain from baseline) criteria were met for the week.

In Study 301, 27% of patients (83/307) on tenapanor met the primary endpoint compared to 19% of patients (56/299) on placebo (p=0.02). In Study 302, 36.5% of patients (107/293) on tenapanor met the primary endpoint compared to 24% of patients (71/300) on placebo (p<0.001). In secondary efficacy analyses of the individual components of the co-primary endpoint, significant improvements in overall CSBM response and overall abdominal pain response were achieved in Study 302. However, in Study 301, efficacy of tenapanor in achieving the endpoint of overall CSBM response (first ranked secondary endpoint) did not achieve

statistical significance; 34% of tenapanor patients (104/307) and 29% of placebo patients (88/299) achieved overall CSBM response (p=0.2).

Analysis of the safety profile of tenapanor reveals that diarrhea is the most prominent adverse effect of tenapanor, occurring in 15% of IBS-C patients across the controlled portions of the 2 confirmatory trials. Diarrhea is an expected pharmacodynamic effect of a drug developed to treat IBS-C. However, in patients at greater risk of complications from diarrhea, such as pediatric and geriatric patients or those with other medical conditions (such as chronic renal disease), diarrhea may be of greater concern. Risk of diarrhea is reflected in labeling.

Less prevalent gastrointestinal AEs, abdominal distension and abnormal gastrointestinal sounds, do not carry significant risk but may be bothersome in IBS patients already suffering from similar symptoms and are noted in the label.

Hyperkalemia was identified by the Applicant as a potential risk in the CKD population, but did not appear to present a major safety concern within the IBS-C trial population. The prevalence of hyperkalemia (as assessed by post-baseline laboratory value shifts) was increased in Study 301 for IBS-C but this finding was not replicated in Study 302. There was no imbalance of AEs related to hyperkalemia in the IBS-C population, and within the subset of IBS-C patients with renal impairment, no increase in hyperkalemia adverse events was reported. However, because there were rare but life-threatening events of hyperkalemia that occurred within the CKD population, the label will reflect this finding

Finally, an increase in the rate of rectal bleeding occurred in patients on tenapanor compared to patients on placebo although the total numbers were small. In Study 301, 4/309 (1.3%) tenapanor patients and 0/301 (0%) placebo patients had AEs of rectal bleeding. In Study 302, 3/293 (1.0%) tenapanor patients and 1/293 (0.33%) placebo patients had AEs of rectal bleeding. Although, the rectal bleeding did not result in hemodynamic instability or significant laboratory changes, colonoscopies were performed in only two cases (one of which was placebo), making it impossible to determine the etiology of the bleeding.

Rectal bleeding was an AE of interest because of published reports of inflammatory bowel disease in patients with inactivating mutations in NHE3 and in NHE3 knock-out animals. Due to concern that patients on tenapanor with rectal bleeding (and some of those with diarrhea) might develop new-onset, inflammatory bowel disease, a post-approval signal detection study within Sentinel is planned.

In conclusion, the benefits of tenapanor, when used at a dosage of 50 mg BID, outweigh its risks for the treatment of adult IBS-C and supports approval of this application.

11. Pediatrics

Under the Pediatric Research Equity Act (PREA) (21 U. S. C. 335), 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. PREA applies to NDA 211801 tenapanor tablet, which is a new molecular entity.

There is an Agreed Initial Pediatric Study Plan (iPSP), dated September 26, 2017, that details the plan to request a partial waiver in pediatric patients from birth to less than 6 years of age because necessary studies are impossible or highly impracticable in the very young. The iPSP also documents a plan (b) (4) Under PREA, the Applicant will be required to conduct a juvenile animal toxicology single-dose study in 4-day old mice and an 8-week, repeat dose study in rats with dosing initiated on PND 21 to support exposure in humans 2 years to less than 12 years. The PREA postmarketing requirements are summarized in Section 14 of this Unireview. A separate Division of Pediatric and Maternal Health Labeling Review for NDA 211801 is archived in DARRTS.

To fulfill their obligation under PREA, the Applicant requested a deferral of pediatric studies at the time of NDA submission (adult studies are complete and the drug is ready for approval) and a partial waiver for conducting studies in pediatric patients less than 6 years of age (studies are impossible or highly impracticable). Acknowledging that similarities in the disease pathophysiology and anticipated response to treatment exist between adults and pediatric patients with IBS (particularly for the adolescent population), partial extrapolation of efficacy is proposed.

The planned post-marketing studies to address pediatric patients are listed in Section 14.

12. Labeling Recommendations

The Applicant's proposed label was reviewed, and revisions and comments have been communicated to the Applicant during labeling negotiations. The final approved labeling will be appended to the approval letter. The major clinical issues related to the label and DGIEP-proposed changes with rationale include the following:

1. Section 5. Warnings and Precautions

   5.1. Pediatrics. The Division recommended strengthening the pediatric warning to a contraindication in patients under age 6 due to Risk of Severe Dehydration in Pediatric Patients, including a summary of animal data showing increased mortality in young juvenile rats (see Section 5.5.5. Juvenile Toxicology Studies). DGIEP further included a statement to avoid the use of tenapanor in pediatric patients 6 to <12years of age.

2. Section 6. Adverse Reactions

   The Division recommended expanding the section on Common Adverse Reactions to include additional common AEs, including abdominal distension, flatulence, and dizziness, which occurred in greater than 2% of tenapanor-treated patients in at least one of the two phase 3 trials, when analyzed separately.

   DGIEP recommended including a section of rare adverse reactions to include: rectal bleeding, elevated ALT, elevated AST and abnormal gastrointestinal sounds.

3. Section 8. Special Populations

   8.1. Pregnancy. Language modified to acknowledge minimal systemic exposure from the to-be-marketed dosage, and to clarify that maternal exposure is not expected to result in fetal exposure to the drug. Summary of nonclinical data from reproductive toxicity studies in both rats and rabbits was included.

   8.4 Pediatric Use. In addition to the strengthened warnings regarding dehydration risk in pediatrics (Section 5 of the label), detailed animal data were provided in this section regarding mortality in young juvenile rats, corresponding to humans less than age 2, on relatively low doses (0.1 mg/kg) of tenapanor.

   8.5 Geriatric Use. This section was changed [redacted] (b) (4) to acknowledge that 100 patients in the controlled phase 3 trials (8% of the study population) was 65 years or older, and no differential efficacy or safety findings were noted. It further acknowledges that greater sensitivity of older individuals to some adverse events cannot be ruled out.

Section 13. Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility. Additional details regarding carcinogenesis and fertility, nonclinical studies, were added and information provided regarding the lack of carcinogenicity of the M1 metabolite. (b) (4)

4. Section 14. Clinical Studies

This section was edited for brevity. Concepts of (b) (4)

13. Risk Evaluation and Mitigation Strategies

Risks identified will be communicated via labeling. No Risk Evaluation and Mitigation Strategies program was required.

14. Postmarketing Requirements and Commitment

A total of 6 post-marketing requirements will be issued. Five of these studies will be issued under PREA, to appropriately evaluate the drug in pediatrics, and an additional study will be conducted to inform the pregnancy/lactation related safety issues.

In IBS, the Division routinely requires pediatric studies down to age 6, and waives requirements for patients <6 years of age, as IBS is rarely diagnosed in that age group, and studies would be impracticable to conduct.

Because of the findings in the juvenile animal studies conducted to date (refer to Section 5) where deaths occurred in young juvenile animals, even at very low doses, a safety margin that would permit initiation of studies in patients < 12 years of age was not identified. It is hypothesized that perhaps the very young animals (human age equivalent <2 years of age) may have a maturation related reason for the apparent increases in susceptibility to toxicity from tenapanor, which may change or improve with advancing age. As a result, a post-marketing requirement will be issued to conduct another juvenile animal toxicology study, with dosing initiated at PND21. The goal of this study will be to determine if older juvenile animals tolerate the drug and if safety margin can be identified that would permit initiation of trials in pediatric patients ages 6 and older.

3583-1 "A 60-day, repeat dose, GLP juvenile animal toxicology study in rats in which the dosing of the animals should be initiated on post-natal day 21"

Studies in patients 6 to <12 years of age will be conducted if the findings from the above juvenile toxicology study support initiation. This will include 2 studies, one for dose-ranging, and a second study to confirm efficacy:

3583-2 "A randomized, double blind, controlled, dose ranging study to assess the safety and efficacy of tenapanor for the treatment of irritable bowel syndrome with constipation (IBS-C) in pediatric patients ages 6 to less than 12 years of age. The study will include at least 2 doses and treatment duration will be 4 weeks".

3583-4 "A randomized, controlled study to assess the safety and efficacy of tenapanor for the treatment of irritable bowel syndrome with constipation (IBS-C) in pediatric patients 6 to less than 12 years of age".

Studies in pediatric patients 12 years of age and older are supported by the nonclinical toxicology studies already conducted, and further supported by the adult experience. There is greater similarity between adolescents and adults with IBS, and therefore, the pediatric study in this age group relies more heavily on extrapolation from adult data. As a result a single, combined study will be conducted in patients 12 to 17 years of age, to assess dose-ranging, safety, efficacy, and PK.

3583-3 "A 12-week, randomized, controlled study to assess the safety and efficacy of tenapanor for the treatment of irritable bowel syndrome with constipation (IBS-C) in pediatric patients 12 to less than 18 years of age. The study will evaluate at least 2 doses."

Because IBS is a chronic condition and patients may be expected to use tenapanor for chronic administration, a safety extension study to collect longer term safety data on chronic administration will be conducted for patients 6 to 17 years of age (which may be combination of exposure from the initial phase 2 or phase 3 trial plus additional time in the extension study).

3583-5 "An open-label, long-term extension study to assess the safety of ongoing treatment with tenapanor for irritable bowel syndrome with constipation (IBS-C) in pediatric patients 6 to less than 18 years of age, who participated in study 3583-2, 2583-3, or 3585-4."

Finally, because the underlying reason for the extreme sensitivity to tenapanor noted in young juvenile animals is not well explained, it presents a concern that if neonates were inadvertently exposed to small amounts of tenapanor in the breast milk from a lactating mother being treated, that it could present a serious safety risk. Although tenapanor is "considered minimally absorbed" a small amount was detected in the serum in some patients. Whether or not any of that small amount could be transferred into the breast milk is unknown. Therefore, a milk-only lactation study will be conducted, to determine whether or not there is any detectable level of tenapanor in the milk, as any detectable drug may alter the acceptability of use of tenapanor while breast feeding.

3583-6: Perform a milk-only lactation trial in lactating women who have received multiple, once daily, doses of tenapanor therapeutically to assess concentrations of tenapanor (and its active metabolite) in breast milk using a validated assay.

15. Associate Division Director (Clinical) Comments

I concur with the recommendation of the review team to approve NDA 211801 for IBSRELA (tenapanor) for the treatment of irritable bowel syndrome with constipation (IBS-C) in adults. Tenapanor is a sodium/hydrogen exchanger 3 (NHE3) inhibitor. Although there are several products that are FDA-approved for treatment of IBS-C (lubiprostone, linaclotide, plecanatide, and tegaserod), tenapanor is the first product to be approved in this class. The recommended dosage is 50 mg twice daily and is intended to be administered immediately prior to breakfast (or first meal of the day) and immediately prior to dinner. Data submitted in the NDA support the conclusion that the benefits of treatment with tenapanor in the intended population outweigh the identified risks. However, because treatment-related mortalities (presumably due to dehydration) were observed in young juvenile rats (less than 1-week old rats; approximate human age equivalent less than 2 years of age) and there are currently no data available in older juvenile rats (human age equivalent 2 years to less than 12 years), a boxed warning will indicate that tenapanor is contraindicated in patients less than 6 years of age and its use should be avoided in patients 6 years to less than 12 years of age. In the absence of older juvenile animal toxicity data and concerning young juvenile animal data, along with lack of clinical data in pediatric patients, the boxed warning will also indicate that the safety and effectiveness of tenapanor in pediatric patients less than 18 years of age have not been established.

I agree with the review team that data submitted in this NDA are adequate to support a conclusion that the effectiveness of tenapanor has been established in the intended adult population. The submission included two adequate and well-controlled trials, and both trials achieved statistical significance on the primary endpoint of “overall responder” for at least 6 of the first 12 weeks. The overall responder endpoint was defined as achieving at least a 30% reduction from baseline in average of the worst daily abdominal pain score and an increase of at least 1 complete spontaneous bowel movement (CSBM) from baseline, both in the same week for at least 6 of the 12 weeks. In the trial that was 26 weeks in duration, the proportion of responders for 13 out of 26 weeks was greater in tenapanor-treated patients compared to placebo-treated patients. Additional secondary endpoints also supported the primary efficacy analysis.

The most common adverse reactions observed in clinical trials include diarrhea, abdominal distension, flatulence and dizziness. Severe diarrhea occurred in 2.5% of tenapanor-treated patients, compared to 0.2% of placebo-treated patients, during controlled trials. Since these patients are at risk of dehydration, the Warnings and Precautions section of labeling will include diarrhea, advising prescribers to suspend dosing in patients who develop severe diarrhea and rehydrate these patients.

Hyperkalemia was noted as a potential risk in another patient population (chronic kidney disease); however, it did not appear to present a major safety concern within the IBS-C trial population based on available data. Because there were rare but life-threatening events of hyperkalemia that occurred within the chronic kidney disease population, labeling will reflect this finding to inform prescribers of this potential risk.

Although only reported in a small proportion of tenapanor-treated IBS-C patients (1% in each controlled trial), rectal bleeding is an adverse event of interest because of published reports of inflammatory bowel disease (IBD) in patients with inactivating mutations in NHE3 and in NHE3 knock-out animals. Due to the concern that patients on tenapanor with rectal bleeding (and some of those with diarrhea) might develop new-onset IBD, assessment of the risk of IBD in the Sentinel's Active Risk Identification and Analysis (ARIA) System is planned.

A REMS will not be required. Post-marketing required studies will assess: 1) the effect of repeat dosing in juvenile rats (dosing to be initiated on postnatal day 21) to support dosing in pediatric patients 6 years and older; 2) multiple doses in a 4-week study (i.e., a dose ranging study) to identify the appropriate dose(s) to study in pediatric patients ages 6 to less than 12 years; 3) the safety and efficacy of tenapanor in pediatric patients with IBS-C ages 6 to less than 12 years; 3) the safety and efficacy of tenapanor in pediatric patients with IBS-C ages 12 to less than 18 years; 4) the long-term safety of ongoing treatment in pediatric patients with IBS-C ages 6 to less than 18 years who completed controlled trials; and 5) the concentrations of tenapanor (and its active metabolite) in breast milk using a validated assay.

16. Appendices

16.1. Clinical Appendices

16.1.1. Narratives of Deaths (ESRD)

Narrative

Case 1: (b) (6) Study D5613C0001
Started on tenapanor 1 mg BID on (b) (6)
Stopped tenapanor 1 mg BID on (b) (6)
Died on (b) (6)

The patient was a 66-year-old, Caucasian male with a history of end-stage renal disease (ESRD) on dialysis with arteriovenous fistula. Co-morbidities included: pulmonary tuberculosis, hypertension, vasculitis, benign prostatic hyperplasia, brachiocephalic vein stenosis, diabetes mellitus, atrial flutter, hyperphosphatemia, coronary artery disease, hiatus hernia, emphysema, venous occlusion and peripheral ischemia.

On (b) (6) (Day 8), the patient had a moderate arteriovenous fistula thrombosis and was hospitalized. He was treated by thrombectomy, the event resolved, and he was discharged the same day. He was noted to have "nonserious" left leg ischemia.

On (b) (6) (Day 20), he was hospitalized for severe left leg ischemia.

On (b) (6), he had a percutaneous angioplasty of the iliac artery.

On (b) (6) (Day 26), he had a recanalization of the left femoral artery and femoral popliteal bypass.

On (b) (6) (Day 28), he had the third digit of his left foot amputated.

On (b) (6) (Day 34), he had partial amputation of his left foot, due to necrosis. His condition worsened and on (b) (6) (Day 43), 23 days after his last study drug dose, he died of heart failure.

Although the immediate cause of death, heart failure, was not a safety concern in any of the trials, the events leading up to this patient's death, which included ischemic peripheral vascular disease of the lower extremity requiring angioplasty, bypass and amputation, were consistent with a potential safety signal for increased risk of ischemic/embolic events seen in the ESRD group (see Section 10.2.12 on special populations). There was no mention of diarrhea and dehydration in this narrative to suggest that hypovolemia could have compromised peripheral circulation.

Narrative

Case 2: (b) (6), Study TEN-02-201
Started on tenapanor 3 mg BID on (b) (6)
Last dose of tenapanor 3 mg BID on (b) (6)
Partial small-bowel obstruction on (b) (6)
Died on (b) (6)

The patient was a 71-year-old, Caucasian female with a history of ESRD on dialysis with arteriovenous graft. Co-morbidities included: cerebrovascular accident with hemiparesis, anxiety, depression, type 2 diabetes mellitus, hypertension, hypothyroidism, intradialytic hypotension. She was status post cholecystectomy.

On (b) (6) (Day 6), the patient presented to the hospital with complaints of nausea, vomiting and abdominal pain. She was found to have a high-grade, partial small bowel obstruction and was admitted to the hospital for treatment with nasogastric tube placement and intravenous fluids. The study drug was interrupted. On (b) (6) (Day 13), her physical examination had normalized, and the patient was discharged from the hospital. The patient resumed study drug on (b) (6) (Day 16).

On (b) (6) (Day 18), the patient presented to the hospital with complaints of nausea and vomiting. She was admitted to the hospital and treated conservatively for a diagnosis of ileus. Study drug was interrupted. On (b) (6) (Day 20), the patient was able to tolerate her usual diet and activity and was discharged from the hospital.

On (b) (6) (Day 22), the patient presented to the hospital with complaints of nausea, vomiting and abdominal pain. She was diagnosed with small bowel obstruction and possible perforation of bowel and she was withdrawn from the study. She was treated with nasogastric tube placement and nothing by mouth. She deteriorated and was transferred to the intensive care unit (ICU) for treatment of "shock." In ICU, she was treated with central line placement, intravenous pressor and continuous renal replacement therapy. She was evaluated by Surgery, who opined that she would be a very high-risk, surgical candidate. The family elected to have her transferred to a long-term, acute care hospital and, subsequently, to hospice. On (b) (6), the patient expired due to small bowel obstruction, worsening of ESRD and perforation of bowel.

The investigator did not find this death from intestinal obstruction and perforation related to the study drug, most likely because the patient's history of prior abdominal surgery is a known risk factor for small intestinal obstruction. It is interesting to note, however, that this was one of two episodes of small intestinal obstruction reported in patients on tenapanor (the second occurred in the IBS-C group) as compared to none on placebo. It may also be significant because other IBS-C drugs, such as linaclotide and lubiprostone, are contraindicated in patients with a known history of obstruction. Finally, the occurrence of a perforation, in addition to obstruction, is noteworthy because of detection of a small increase in the rate of intra-abdominal surgical emergencies in IBS-C patients on tenapanor to be discussed below in Serious Adverse Events. Because this patient did not undergo surgical intervention or autopsy, the cause of her obstruction and perforation will remain unknown.

16.1.2. Schedule of Assessments

Table 57. Adverse Event and Vital Sign Assessments by Treatment Visit, Integrated Safety Analysis Set

Evaluation	Screening	Treatment Period											
Study Week (s)		0	2	3	4	6	8	12	14	16	20	26	39
D5612C00001	-2		X		X		X	X*		X			
RDX5791-201	-1		X	X	X*	X							
TEN-01-301	-2		X		X		X	X	X	X*			
TEN-01-302	-2		X		X		X	X		X	X	X*	
TEN-01-303								X				X*	X*

* Last dose of study drug
Source: Reviewer's table, adapted from Applicant's Schedules of Assessments, submitted September 12, 2018

Table 58. Schedule of Assessments - Study 301

Evaluation	Screen	Treatment Period					Randomized Withdrawal Period	
Site Visit	1	2	3	4	5	6	7	8/ET[h]
Study Day(s)	-14	1 ± 3	15 ± 3	29 ± 3	57 ± 3	85 ± 3	99 ± 3	113 ± 3
Informed Consent[a]	X							
Inclusion/Exclusion	X	X						
Demographics	X							
Medical History (including GI history)	X	X[b]						
Prior/Concomitant Medications	X	X	X	X	X	X	X	X
Physical Examination	X					X		X
Vital Signs[c]	X	X	X	X	X	X	X	X
Height	X							
Safety Laboratory Tests	X			X		X		X
Pharmacogenomics Blood Sample			X[g]	X[g]	X[g]	X[g]	X[g]	X[g]
Biomarker Sample	X					X		X
FSH Test[d]	X							
Serology	X							
Urine Pregnancy Test[d]	X	X		X		X		X
Urinalysis	X			X		X		X
12-Lead Electronic ECG	X					X		X
IVRS Training/Compliance Check/Reminder	X	X	X	X	X	X	X	
IBS-QOL PRO		X				X		X

Evaluation	Screen	Treatment Period					Randomized Withdrawal Period	
Site Visit	1	2	3	4	5	6	7	8/ET[h]
Study Day(s)	-14	1 ± 3	15 ± 3	29 ± 3	57 ± 3	85 ± 3	99 ± 3	113 ± 3
Treatment Satisfaction PRO				X	X	X		X
Randomization		X						
Randomization #2[f]						X		
Daily PROs[e]	X	X	X	X	X	X	X	X
Drug Dispensed/Returned		D		D/R	D/R	D/R		R
Adverse Event Assessments		X	X	X	X	X	X	X

Abbreviations: BID = Twice daily; BM = Bowel movement; BSFS = Bristol stool form scale; ECG = Electrocardiogram; ET = Early Termination; FSH = Follicle-stimulating hormone; GI = Gastrointestinal; IBS = Irritable bowel syndrome; IVRS = Interactive voice response system; QOL = Quality of life; PRO = Patient reported outcomes.

[a] The ICF must have been signed before any study procedures were performed; The ICF may have been signed before the Screening Visit.

[b] Medical history for Visit 2; recorded only changes to Medical history from Visit 1.

[c] Vital signs included systolic and diastolic blood pressure (seated), heart rate, respiratory rate, body temperature and body weight.

[d] FSH was assessed in post-menopausal women (at screening only); pregnancy tests were performed on all females <60 years of age unless there was a documented method of sterilization, or the FSH test confirmed post-menopausal status. A positive urine pregnancy test was to be verified with a serum pregnancy test.

[e] Daily PROs were collected via the IVRS diary and included the following: frequency and time of each BM, sensation of complete bowel emptying, stool consistency (measured using the BSFS) of each BM, straining, abdominal pain, abdominal discomfort, abdominal bloating, abdominal fullness, abdominal cramping, use of and time of rescue medication, IBS severity (weekly), constipation severity (weekly), adequate relief of IBS symptoms (weekly, after randomization), degree of relief of IBS symptoms (weekly, after randomization).

[f] All subjects who received Placebo were switched to tenapanor 50 mg BID; Subjects who received tenapanor 50 mg BID were randomized 1:1 to receive either tenapanor 50 mg BID or Placebo 50 mg BID.

[g] The pharmacogenomics sample was optional and required a subject to specify acceptability on the informed consent. If a subject opted in, the blood sample could have been taken at any one visit after randomization.

[h] Procedures described for Visit 8/ET should have been performed on all subjects who withdrew early from the study, if possible.

Source: Sponsor's Study Report Pages 29 and 30 of 1530

Table 59. Schedule of Assessments - Study 302

Evaluation	Screen	Treatment Period							
Site Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9/ET[f]**
Study Week	**-2**	**0**	**2**	**4**	**8**	**12**	**16**	**20**	**26**
Study Day(s)	**-14**	**1**	**15±3**	**29±3**	**57±3**	**85±3**	**113±3**	**141±3**	**183±3**
Informed consent[a]	X								
Inclusion/exclusion	X	X							
Demographics	X								
Medical history (including GI history)	X	X[b]							
Prior/concomitant medications	X	X	X	X	X	X	X	X	X
Physical exam	X								X
Vital signs[c]	X	X	X	X	X	X	X	X	X
Height	X								
Serum chemistry and hematology	X			X		X			X
Pharmacogenomics sample[g]				X		X			X
Biomarker sample	X			X		X			X
FSH test[d]	X								
Serology	X								
Urine pregnancy test[d]	X	X		X		X			X
Urinalysis	X			X		X			X
12-lead electronic ECG	X					X			X
IVRS training/compliance check and reminder	X	X	X	X	X	X	X	X	
IBS-QoL PRO		X				X			X

Evaluation	Screen	Treatment Period							
Site Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9/ET[f]**
Study Week	**-2**	**0**	**2**	**4**	**8**	**12**	**16**	**20**	**26**
Study Day(s)	**-14**	**1**	**15±3**	**29±3**	**57±3**	**85±3**	**113±3**	**141±3**	**183±3**
Treatment satisfaction PRO				X	X	X	X	X	X
Randomization		X							
Daily PROs[e]	X	X	X	X	X	X	X	X	X
Drug dispensed/returned		D		D/R	D/R	D/R	D/R	D[h]/R	R
Adverse event assessments			X	X	X	X	X	X	X

Abbreviations: BM = Bowel movement; BSFS = Bristol stool form scale; D = Dispensed; ECG = Electrocardiogram; ET = End of treatment; FSH = Follicle stimulating hormone; GI = Gastrointestinal; IBS-QoL = Irritable bowel syndrome quality of life; ICF = Informed consent form; IVRS = Interactive voice response system; PRO = Patient reported outcomes; R = Returned.

[a] The ICF must have been signed before any study procedures were performed and prior to discontinuing any prohibited medications; the ICF may have been signed before the Screening visit.

[b] Medical history for Visit 2; recorded only changes to medical history from Visit 1.

[c] Vital signs included systolic and diastolic blood pressure (seated), heart rate, respiratory rate, body temperature and body weight.

[d] FSH was assessed in post-menopausal women (at screening only); pregnancy tests were performed on all females <60 years of age unless there was a documented method of sterilization, or the FSH test confirmed post-menopausal status.

[e] Daily PROs were collected via the IVRS diary and included the following: frequency and time of each BM, sensation of complete bowel emptying, stool consistency (measured using the BSFS) of each BM, straining, abdominal pain, abdominal discomfort, abdominal bloating, abdominal fullness, abdominal cramping, use of and time of rescue medication, IBS severity (weekly), constipation severity (weekly), adequate relief of IBS symptoms (weekly, after randomization), and degree of relief of IBS symptoms (weekly, after randomization).

[f] All ET procedures listed for Visit 9 should have been performed on any subject that terminated the study early.

[g] The pharmacogenomics sample was optional and required a subject to specify acceptability on the informed consent. If a subject opted in, the blood sample could have been taken at any 1 visit after randomization with a scheduled blood draw (Visits 4, 6, or 9).

[h] Two bottles were dispensed at this visit.

Source: Sponsor's Study Report Pages 26 and 27 of 1738

16.1.3. Integrated Safety Set Analyses

Serious Adverse Events in the Integrated Safety Analysis Set[6]

SAEs occurred infrequently in tenapanor-treated patients and overall rates did not differ between treatment arms. In total, 18 SAEs occurred in 18/1343 patients (1.3%, 3.9/100 PY) receiving tenapanor and 11 SAEs occurred in 8/738 (1.1%, 4.1/100 PY) of patients receiving placebo. In this safety population, there was no clear dose-response for SAEs. SAEs were reported in 4/134 (3.0%) of patients on 10 mg tenapanor, 1/136 (0.74%) of patients on 30 mg tenapanor, 13/1073 (1.2%) of patients receiving tenapanor 100 mg daily, and 8/738 (1.1%) of patients on placebo. Of note, interpretation of dose comparisons from integrated analyses is potentially limited by differences in duration of follow-up across arms.

Our analysis revealed that no organ system or preferred term diagnosis was overrepresented for SAEs in either treatment group (Table 60). Of note, however, four patients on tenapanor (0.3%, 0.87/100 PY) had abdominal surgical emergencies (appendicitis, cholecystitis, small intestinal obstruction, and endometriosis requiring emergency hysterectomy) as compared to one placebo (0.14%, 0.4/100 PY) patient (ovarian vein thrombosis).

Given that the drug is locally acting with minimal systemic absorption, the Applicant's assertion that the majority of the SAE events are unrelated is reasonable. Gastrointestinal tract AEs due to local effect would be anticipated to occur and may potentially be drug related.

[6] This Analysis Set corresponds to the Reviewer's Integrated Safety Analysis Set defined in Table 31. It combines data from the phase 2 and phase 3 trials conducted in IBS-C patients.

Table 60. Serious AEs by Diagnosis and Treatment Group, Integrated Safety Analysis Set

	Treatment Group	
	Tenapanor, N=1343	**Placebo, N=738**
AE Diagnosis (SOC and PT)	***456 PY***	***242 PY***
Gastrointestinal Disorders		
Abdominal pain	1(0.07%)	1 (0.14%)
Diarrhea	1 (0.07%)	0
Lower gastrointestinal hemorrhage	0	1 (0.14%)
Nausea	1 (0.07%)	1 (0.14%)
Small intestinal obstruction	1 (0.07%)	0
Vomiting	0	1 (0.14%)
TOTAL SERIOUS GASTROINTESTINAL AEs	***4 (0.30%)***	***4 (0.55%)***
	0.87/100 PY	***1.7/100 PY***
Blood and lymphatic system disorders		
Leukocytosis	1 (0.07%)	0
Ear and labyrinth disorders		
Vertigo	0	1 (0.14%)
General disorders and administration site conditions		
Systemic Inflammatory Response	0	1 (0.14%)
Hepatobiliary disorders		
Cholecystitis	1 (0.07%)	0
Infections and infestation		
Appendicitis	1 (0.07%)	0
Osteomyelitis	0	1 (0.14%)
Pyelonephritis	0	1 (0.14%)
Urinary tract infection	1 (0.07%)	0
Injury, poisonings and procedural complications		
Abdominal injury with cervical, vertebral and rib fracture	1 (0.07%)	0
Musculoskeletal and connective tissue disorders		
Osteoarthritis	1 (0.07%)	0
Neoplasms, benign, malignant and unspecified		
Laryngeal neoplasm	1 (0.07%)	0
Pineal tumor	1 (0.07%)	0
Nervous system disorders		
Migraine	1 (0.07%)	0
Status migrainosus	1 (0.07%)	0
Psychiatric disorders		
Alcohol withdrawal	1 (0.07%)	0
Major depression/panic attack	1 (0.07%)	0
Panic attack	1 (0.07%)	0
Reproductive system and breast disorders		
Endometriosis	1 (0.07%)	0
Ovarian vein thrombosis	0	1 (0.14%)
Vascular disorders		
Carotid artery occlusion	0	1 (0.14%)
TOTAL SERIOUS AEs	***18 (1.3%)***	***10 (1.4%)***
	3.9/100 PY	***4.1/100 PY***

Abbreviations: AE, adverse event; PT, preferred term; SOC, system organ class
Source: Reviewer's table, adapted from Applicant's Table 4-15, Integrated Summary of Safety (ISS), p. 142, submitted September 12, 2018

Adverse Events of Severe Intensity in the Integrated Safety Analysis Set

Adverse events rated as "severe" in intensity occurred at relatively low rates in tenapanor-treated IBS-C patients; 49/1343 (3.6%, 11/100 PY) of tenapanor-treated patients and 13/738 (1.8%, 5.4/100 PY) of placebo-treated patients had 58 and 16 severe adverse events, respectively. The most common severe AEs were gastrointestinal occurring with a cumulative incidence rate of 2.6% (7.7/100 PY) in tenapanor-treated patients and 0.5% (1.7/100 PY) in placebo-treated patients (Table 61). Of these, the majority were diarrhea, occurring at a rate of 1.7% (5.0/100 PY) in tenapanor-treated patients and 0.14% (0.4/100 PY) in placebo-treated patients. The gastrointestinal, severe adverse events of diarrhea were judged by the investigator (and FDA) to be related to study drug. Severe intensity adverse events in other system organ classes were infrequent and occurred in similar proportion between drug and placebo arms.

Table 61. Severe AEs by Diagnosis and Treatment, Integrated Safety Analysis Set

	Treatment Group	
	Tenapanor	Placebo
	N=1343	N=738
AE Diagnosis (SOC and PT)	*456 PY*	*242 PY*
Gastrointestinal Disorders		
Diarrhea	23 (1.7%)	1 (0.14%)
	5/100 PY	*0.41/100 PY*
Abdominal pain	5 (0.30%)	2 (0.28%)
	1/100 PY	*0.82/100 PY*
Abdominal distension	3 (0.22%)	0
	0.66/100 PY	*0/100 PY*
Abdominal hernia	0	1 (0.14%)
	0/100 PY	*0.41/100 PY*
Nausea	2 (0.15%)	0
	0.44/100 PY	
Small intestinal obstruction	1 (0.07%)	0
	0.22/100 PY	
Vomiting	1 (0.07%)	0
TOTAL SEVERE GASTROINTESTINAL AEs	***35 (2.6%)***	***4 (0.54%)***
	7.7/100 PY	***1.7/100 PY***
General disorders and administration site reactions		
Intolerance to metronidazole	1 (0.07%)	0
Systemic inflammatory response syndrome	0	1 (0.14%)
Hepatobiliary disorders		
Cholecystitis	1 (0.07%)	0
Infections and infestation		
Appendicitis	1 (0.07%)	0
Osteomyelitis	0	1 (0.14%)
Pyelonephritis	0	1 (0.14%)
Tooth abscess	1 (0.07%)	0
Tooth infection	1 (0.07%)	
Urinary tract infection	0	1 (0.14%)
Injury, poisonings and procedural complications		
Abdominal injury with cervical, vertebral, and rib fracture	1 (0.07%)	0
Tooth fracture	1 (0.07%)	0

	Treatment Group	
	Tenapanor	Placebo
	N=1343	N=738
AE Diagnosis (SOC and PT)	*456 PY*	*242 PY*
Investigations		
Alanine aminotransferase increased	1 (0.07%)	
Aspartate aminotransferase	1 (0.07%)	
Blood lactate dehydrogenase increased	1 (0.07%)	
Musculoskeletal and connective tissue disorders		
Back pain	1 (0.07%)	0
Left flank pain	1 (0.07%)	
Lumbar spasm	0	1 (0.14%)
Osteoarthritis	1 (0.07%)	0
Rotator cuff syndrome	1 (0.07%)	0
Sciatica	1 (0.07%)	0
Neoplasms, benign, malignant and unspecified		
Basal cell carcinoma	0	1 (0.14%)
Laryngeal neoplasm	1 (0.07%)	0
Nervous system disorders		
Headache	1 (0.07%)	0
Migraine	1 (0.07%)	0
Psychiatric disorders		
Affective disorder	0	1 (0.14%)
Anxiety	0	1 (0.14%)
Depression	1 (0.07%)	0
Panic attack	1 (0.07%)	0
Renal and urinary system disorders		
Nephrolithiasis	0	1 (0.14%)
Respiratory and mediastinal disorders		
Chronic obstructive pulmonary disease	0	1 (0.14%)
Pulmonary embolism	0	1 (0.14%)
Upper Respiratory Infection	1 (0.07%)	
Reproductive system and breast disorders		
Endometriosis	1 (0.07%)	0
Ovarian vein thrombosis		1 (0.14%)
Skin and subcutaneous disorders		
Rash morbilliform	1 (0.07%)	0
TOTAL SEVERE AEs	***58 (4.3%)***	***16 (2.2%)***
	13/100 PY	*6.6/100 PY*

Abbreviations: AE, adverse event; PT, preferred term; SOC, system organ class
Source: FDA's table, adapted from Applicant's Adverse Event Listings, Studies D5612C00001, RDX5791-201, TEN-01-301, TEN-01-302, submitted September 12, 2018

Additional Analyses of Changes in Liver Enzymes in Integrated Safety Analysis Set

Table 62. Abnormal Alanine Aminotransferase Shifts From Baseline, Integrated Safety Analysis Set

	Treatment Arm	
Highest Postrandomization Value	**Tenapanor (N=1532 Patients*) *456 PY***	**Placebo (N=848 Patients*) *242 PY***
Normal ALT at baseline	N=1488 patients	N=823 patients
ALT >2X ULN, ≤3X ULN	1 (0.07%)	0
ALT >3X ULN, ≤5X ULN	2 (0.13%)	0
ALT >5X ULN, ≤10X ULN	0	0
ALT >10X ULN, ≤15X ULN	0	0
Abnormal ALT at baseline^	N=44 patients	N=25 patients
ALT >2X ULN, ≤3X ULN	5 (11%)	1 (4.0%)
ALT >3X ULN, ≤5X ULN	0	0
ALT >5X ULN, ≤10X ULN	0	0
ALT >10X ULN, ≤15X ULN	0	0
Total ALT elevation	8 (0.52%)	1 (0.11%)
	1.8/100 PY	*0.4/100 PY*

* Total number of patients for whom evaluable data was available
^ includes only patients whose postbaseline value was higher than baseline abnormal.
Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal
Source: Reviewer's table, derived from Applicant's LB STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

Table 63. Abnormal Aspartate Aminotransferase Shifts From Baseline, Integrated Safety Analysis Set

	Treatment Arm	
Highest Postrandomization Value	**Tenapanor (N=1532*) *456 PY***	**Placebo (N=848*) *242 PY***
Normal AST at baseline	N=1488 patients	N=823 patients
AST >2X ULN, ≤3X ULN	3 (0.21%)	3 (0.36%)
AST >3X ULN, ≤5X ULN	3 (0.21%)	0
AST >5X ULN, ≤10X ULN	0	3 (0.36%)
AST >10X ULN, ≤15X ULN	0	0
Abnormal AST at baseline	N=44 patients	N=25 patients
AST >2X ULN, ≤3X ULN	6 (14%)	4 (16%)
AST >3X ULN, ≤5X ULN	1 (2.3%)	0
AST >5X ULN, ≤10X ULN	2 (4.6%)	0
AST >10X ULN, ≤15X ULN	0	0
Total AST elevation	15 (1.0%)	10 (1.2%)
	3.3/100 PY	*4.1/100 PY*

* Total number of patients for whom evaluable data was available
Abbreviations: AST, aspartate aminotransferase; ULN, upper limit of normal
Source: Reviewer's table, derived from Applicant's LB STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

Table 64. Abnormal Total Bilirubin Shifts From Baseline, Integrated Safety Analysis Set

	Treatment Arm	
	Tenapanor (N=1532*)	**Placebo (N=848*)**
Highest Postrandomization Value	*456 PY*	*242 PY*
Normal BILI at baseline	N=1488	N=832
BILI >2X ULN, ≤3X ULN	1 (0.07%)	0
BILI >3X ULN, ≤5X ULN	0	0
BILI >5X ULN, ≤10X ULN	0	0
BILI >10X ULN, ≤15X ULN	0	0
Abnormal BILI at baseline		
BILI >2X ULN, ≤3X ULN	0	0
BILI >3X ULN, ≤5X ULN	0	0
BILI >5X ULN, ≤10X ULN	0	0
BILI >10X ULN, ≤15X ULN	0	0
Total BILI elevation	1 (0.07%)	0
	2.2/100 PY	*0/100 PY*

* Total number of patients for whom evaluable data was available.
Abbreviations: BILI, bilirubin; ULN, upper limit of normal
Source: FDA's table, derived from Applicant's Lb STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

Additional Analyses of Hyperkalemia in the Integrated Safety Analysis Set

Table 65. Abnormal Total Potassium Shifts From Baseline, Integrated Safety Analysis Set

	Treatment Arm		
	Tenapanor (N=1333*)	**Placebo (N=864*)**	
Degree of Shift and Baseline Level	*456 PY*	*242 PY*	**CTCAE Grade**
Highest postrandomization value (normal at beginning of study)			
K >7 (~1.4 ULN)	1 (0.07%)	1 (0.12%)	4
K >6 (~1.2 ULN) ≤7	8 (0.60%)	2 (0.23%)	3
K >5.5 (~1.1 ULN) ≤6	15 (1.1%)	7 (0.81%)	2
K>5.0 ≤5.5	69 (5.2%)	49 (5.7%)	1
Highest postrandomization value (high at beginning of study)			
K >7	0	0	4
K >6, ≤7	0 (0%)	1 (0.12%)	3
K >5.5, ≤6	1 (0.07%)	2 (0.23%)	2
K >5.0, ≤5.5	6 (0.45%)	3 (0.35%)	1
Total K elevation (>5.5), all patients	25 (1.9%)	13 (1.5%)	All
	5.5/100 PY	*5.4/100 PY*	
Total K elevation (>5), all patients	100 (7.5%)	65 (7.5%)	All
	22/100 PY	*27/100 PY*	

* Denominator represents all patients in whom a postbaseline K was obtained.
Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; K, potassium; ULN, upper limit of normal
Source: Reviewer's table, derived from Applicant's LB STDM data set and ADSL data set, submitted September 12, 2018, analyzed with JMP 13.0 software

Additional Analyses of Adverse Event rates by Demographic Subgroups

Table 66. SAEs and Severe AEs by Treatment Arm and Demographic Group within Integrated Safety Analysis Set

No. of Patients and Exposure	Safety Population on Tenapanor N=1343 *456 PY*	Safety Population on Placebo N=738 *242 PY*	SAEs on Tenapanor	SAEs on Placebo	SAE Risk Difference (% and incidence rates, 95% CI)	Severe AEs on Tenapanor	Severe AEs on Placebo	Severe AE Risk Difference (% and incidence rates, 95% CI)
Sex								
Male	227 *82 PY*	124 *41 PY*	4 (1.8%) *4.9/100 PY*	0 (0%) *0/100 PY*	**1.8** **[-0.2,3.8]** ***4.9/100 PY***	5 (2.2%) *6.1/100 PY*	0 (0%) *0/100 PY*	**2.2** **[-0.8,5.2]** ***6.1/100 PY***
Female	1116 *374 PY*	614 *201 PY*	14 (1.3%) *3.7/100 PY*	8 (1.3%) *4.0/100 PY*	**0** ***-0.3/100 PY***	45 (4.0%) *12/100 PY*	13 (2.1%) *6.5/100 PY*	**1.9** **[-0.1,3.9]** ***5.5/100 PY***
Age								
≤65	1251 *414 PY*	683 *223 PY*	16 (1.3%) *3.9/100 PY*	7 (1.0%) *3.1/100 PY*	**0.3** **[-0.7,1.3]** ***0.8/100 PY***	44 (3.5%) *11/100 PY*	13 (1.9%) *5.8/100 PY*	**1.6** **[0.6,2.6]** ***5.2/100 PY***
>65	92 *43 PY*	55 *19 PY*	2 (2.2%) *4.7/100 PY*	1 (1.8%) *5.3/100 PY*	**0.4** **[-4.6,5.4]** ***-0.6/100 PY***	6 (6.5%) *14/100 PY*	0 (0%) *0/100 PY*	**6.5** **[-2.5,14.5]** ***14/100 PY***
Race								
White	916 *309 PY*	479 *153 PY*	15 (1.6%) *4.9/100 PY*	4 (0.8%) *2.6/100 PY*	**0.8** **[-0.2,1.8]** ***2.3/100 PY***	38 (4.1%) *12/100 PY*	11 (2.2%) *7.2/100 PY*	**1.9** **[-0.2,3.9]** ***4.8/100 PY***
Black	371 *130 PY*	226 *78 PY*	2 (0.53%) *1.5/100 PY*	4 (1.8%) *5.1/100 PY*	**-1.3** **[-3.3,0.7]** ***-3.6/100 PY***	12 (3.2%) *9.2/100 PY*	2 (0.88%) *2.6/100 PY*	**2.3** **[-0.7,5.3]** ***6.6/100 PY***
Asian	33 *10 PY*	15 *6.1 PY*	0 (0%) *0/100 PY*	0 (0%) *0/100 PY*	**0** ***0/100 PY***	0 (0%) *0/100 PY*	0 (0%) *0/100 PY*	**0** ***0/100 PY***
Native American, Hawaiian	3 *1.3 PY*	2 *0.62 PY*	0 (0%) *0/100 PY*	0 (0%) *0/100 PY*	**0** ***0/100 PY***	0 (0%) *0/100 PY*	0 (0%) *0/100 PY*	**0** ***0/100 PY***
Other, multiple, unknown	20 *6 PY*	16 *5.2 PY*	1 (5%) *17/100 PY*	0 (0%) *0/100 PY*	**5** **[-5,15]** ***17/100 PY***	0 (0%) *0/100 PY*	0 (0%) *0/100 PY*	**0** ***0/100 PY***

Abbreviations: AE, adverse event; SAE, serious adverse event; TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from Applicant's ADSL and AE data files for Studies, D5612C0001, RDX-5792-201,TEN-01-301, TEN-01-302, TEN-01-303, submitted September 12, 2018

Table 67. Demographics of Patients with Rates of Any TEAEs and Diarrhea by Treatment Arm (Integrated Safety Analysis Set)

No. of Patients and Exposure	Safety Population on Tenapanor N=1343 *456 PY*	Safety Population on Placebo N=738 *242 PY*	TEAEs on Tenapanor	TEAEs on Placebo	TEAE Risk Difference Placebo/ Tenapanor (% and Incidence Rates [95% CI])	Diarrhea on Tenapnaor	Diarrhea on Placebo	Diarrhea Risk Difference Placebo/ Tenapanor (% and incidence rates,95% CI)
Sex								
Male	227 *82 PY*	124 *41 PY*	80 (35%) *98/100 PY*	32 (26%) *78/100 PY*	**9** **[-1,19]** ***20/100 PY***	21 (9.2%) *26/100 PY*	0 (0%) *0/100 PY*	**9.2** **[3.2,15]** ***26/100 PY***
Female	1116 *374 PY*	614 *201 PY*	529 (47%) *141/100 PY*	230 (37%) *114/100 PY*	**10** **[5,15]** ***27/100 PY***	161 (14%) *43/100 PY*	17 (2.8%) *8.5/100 PY*	**11** **[8,14]** ***34/100 PY***
Age								
≤65	1251 *414 PY*	683 *223 PY*	556 (44%) *134/100 PY*	246 (36%) *110/100 PY*	**8** **[3,13]** ***24/100 PY***	166 (13%) *40/100 PY*	17 (2.5%) *7.6/100 PY*	**10** **[7,13]** ***32/100 PY***
>65	92 *43 PY*	55 *19 PY*	53 (58%) *123/100 PY*	16 (29%) *84/100 PY*	**29** **[13,45]** ***39/100 PY***	16 (17%) *37/100 PY*	0 (0%) *0/100 PY*	**17** **[4,30]** ***37/100 PY***
Race								
White	916 *309 PY*	479 *153 PY*	429 (47%) *139/100 PY*	169 (35%) *110/100 PY*	**12** **[7,17]** ***29/100 PY***	143 (16%) *46/100 PY*	11 (2.2%) *7.2/100 PY*	**14** **[10,18]** ***39/100 PY***
Black	371 *130 PY*	226 *78 PY*	158 (43%) *122/100 PY*	81 (36%) *104/100 PY*	**7** **[-1,15]** ***18/100 PY***	35 (9.7%) *27/100 PY*	5 (2.2%) *6.4/100 PY*	**7** **[2,12]** ***21/100 PY***
Asian	33 *10 PY*	15 *6.1 PY*	10 (30%) *100/100 PY*	6 (40%) *98/100 PY*	**-10** **[-39,29]** ***2/100 PY***	2 (6.1%) *20/100 PY*	1 (6.2%) *16/100 PY*	**0.1** **[-7.9,8.1]** ***4/100 PY***
Native American, Hawaiian	3 *1.3 PY*	2 *0.62 PY*	1 (33% *77/100/PY*	0 (0%) *0/100 PY*	**33** **[-20,86]** ***77/100 PY***	0 (0%) *0/100 PY*	0 (0%) *0/100 PY*	**0** ***0/100 PY***
Other, multiple, unknown	20 *6 PY*	16 *5.2 PY*	11 (55%) *183/100 PY*	6 (37.5%) *115/100 PY*	**17** **[-15,49]** ***68/100 PY***	2 (10%) *33/100 PY*	0 (0%) *0/100 PY*	**10** **[-10,30]** ***33/100 PY***

Abbreviations: AE, adverse event; SAE, serious adverse event; TEAE, treatment-emergent adverse event
Source: Reviewer's table, derived from Applicant's ADSL and AE data files for Studies, D5612C0001, RDX-5792-201,TEN-01-301, TEN-01-302, TEN-01-303, submitted September 12, 2018

16.2. Nonclinical Pharmacology/Toxicology Appendices

Pharmacology

Study: Gastrointestinal Propulsion Assay of Orally Administered RDX5791 in the Rat (Study # RDX5791-SP-04)

RDX5791 was administered to male SD rats orally at dose levels of 0, 100, 300 or 1000 mg/kg. Thirty minutes following the test article or vehicle administration, 10% suspension of activated charcoal (in 0.25% methylcellulose) was administered orally (10 mL/kg). The rats were euthanized 30 minutes after administration of charcoal, and the intestines removed and placed on a moist paper. The length of the intestine and the distance traveled by the charcoal as a fraction of that length were evaluated for each rat. The result showed that oral administration of RDX5791 at 100, 300, and 1000 mg/kg increased the gastrointestinal motility by 0%, 3%, and 2%, respectively, compared to the vehicle control group. However, these changes were not statistically significant.

Study: A Pharmacodynamic Study of RDX5791 in Cynomolgus Monkeys (Study # RDX5791-EF-06-00)

RDX5791 was administered orally to Cynomolgus monkeys at a dose level of 50 mg/kg BID for 5 days. In a second phase, after a weeklong washout period, animals were orally administered RDX5791 at 150 mg/kg BID for 5 days. Urinary and fecal sodium (Na^+) and potassium (K^+) were determined in samples collected throughout the study period. Soft feces and reduced urinary Na were observed at both doses.

Study: The Effect of RDX5791 on Acute Stress-Induced Visceral Hypersensitivity in Female Rats: A Model of IBS-C (Study # RDX5791-EF -05)

The aim of the study was to evaluate the influence of RDX5791 on colorectal sensitivity in basal condition and in stress-induced visceral hyperalgesia in rats. Six groups of eight to 10 female Wister rats were surgically prepared for electromyography of abdominal cramps in response to colorectal distension. Colorectal distension (CRD) was performed with a balloon inflated for 5 minutes from 0 to 60 mm Hg. Rats were assessed to CRD, 1 day before (basal condition) and 15 minutes after a 2-hour stress session. Rats were treated with either RDX5791 (3, 10, 30, and 50 mg/kg orally) or vehicle (1 mL orally) 1 hour before distension. In basal condition, RDX5791 at all doses (3, 10, 30 and 50 mg/kg) had no effect on colorectal sensitivity or changes in intestinal volumes induced by CRD in comparison to vehicle treated rats. However, after stress, RDX5791 at 30 and 50 mg/kg doses were able to reduce or abolish stress-induced abdominal responses at all distended pressures. Rats at the two lowest doses (3 and 10 mg/kg) had no significant effect. RDX5791 at 30 and 50 mg/kg was able to reduce stress-induced colorectal hypersensitivity to CRD.

Study: Tenapanor in a Mouse Model of Opioid-Induced Constipation (Study # RDX5791-EF-14.00)

The effect of RDX5791 was determined on opioid-induced constipation in mice following administration of a 5% charcoal suspension. Five minutes after charcoal administration, morphine (5 mg/kg) was administered subcutaneously and RDX5791 (10 mg/kg) or vehicle was co-administered orally. Animals were sacrificed 120 minutes after charcoal administration, the intestines removed, and the length of the gut (small intestine and large intestine) in cm as well as extent of charcoal movement from the pylorus to the beginning of the charcoal column in cm was measured. Oral administration of RDX5791 showed a significant effect on gastrointestinal motility in morphine-treated mice.

Study: Tenapanor Reduces Irritable Bowel Syndrome (IBS) Pain Through Inhibition of TRPV1-Dependent Neuronal Hyperexcitability In Vivo (Study # 1920IBS)

A neonatal colorectal irritation model of IBS-like colonic hypersensitivity was generated by intracolonic infusion of 0.5% acetic acid (200 µL) in 10-day old SD rats. Non-sensitized controls received 0.5% saline. Sensitized rats were treated either with the vehicle or tenapanor (0.5 mg/kg), and the nonsensitized controls were treated with vehicle for 7 days. A laxative control group of sensitized rats received PEG 3350 (1000 mg/kg) twice daily. Stools were collected for 5h on the last day of treatment, and wet and dry stool weights were measured to calculate stool water content. Visceral motor reflex (VMR) response to colorectal distension was assessed by electromyogram 1 day after end of the treatment. The VMR to CRD was recorded at 20, 40, 60, and 80 mm Hg pressure applied to the balloon. Colon-specific dorsal root ganglia sensory neurons of adult sensitized rats and nonsensitized controls were labeled in vivo by injecting FAST DiI (1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate), 10 mg/mL in methanol. The excitability and capsaicin response of DiI-positive dorsal root ganglia neurons were assessed by single cell patch clamp assay.

Tenapanor-treated sensitized rats had significantly increased wet and dry stool weights compared with vehicle- and PEG-treated sensitized rats and vehicle-treated nonsensitized controls. Stool water content was significantly higher in tenapanor-treated sensitized rats compared with vehicle-treated sensitized rats and vehicle-treated nonsensitized controls. Tenapanor treatment in sensitized rats significantly reduced VMR responses to CRD compared with vehicle-treated-sensitized rats. Treatment with tenapanor resulted in similar VMR responses to CRD in vehicle-treated nonsensitized controls, suggesting that tenapanor prevents visceral hyperalgesia. Tenapanor treatment significantly reduced the hyperexcitability of colon-specific dorsal root ganglia compared with vehicle treatment in sensitized rats, but had no effect in nonsensitized controls.

Study: Cross-Species Analysis of Intestinal NHE3 and ENaC Gene Expression (Study # RDX5791-EF-15)

The aim of this study was to determine the baseline gene expression levels of NHE3 (*SLC9A3*) and ENaC (epithelial sodium channel; *SCNN1A*, *SCNN1B*, *SCNN1G*) in the intestines of the rat, dog, and human to find if the differences in gene expression could explain the differences in the tolerability of tenapanor. Rats are highly susceptible to intestinal fluid retention and diarrhea,

and have less tolerability to repeated doses of tenapanor than dogs (rat NOAEL: <30 mg/kg/day from a 28-day study and 3 and 10 mg/kg /day for male and female from 6-month study; dog NOAEL: 1000 mg/kg/day from 28-day and 9-month studies). *SLC9A3* (NHE3) expression was observed along the entire intestinal tract in the rat, dog, and human. NHE3 expression levels were similar across all three species throughout the intestine with a few segment specific differences noted. For example, rats had higher expression of NHE3 in the proximal colon relative to dogs and humans, while dogs had lower expression in the duodenum relative to humans. Rats had higher relative NHE3 expression in all intestinal segments other than the distal colon relative to dogs. ENaC expression was lowest in the dog colon compared to human and rat. Since higher levels of functional ENaC would help drive sodium and water absorption, ENaC expression differences are unlikely to explain the improved tenapanor tolerability in dogs relative to rats.

Safety Pharmacology

Study: Neuropharmacological Profile (NPP) of RDX5791 in Rats (Study # RDX5791-SP-02)

RDX5791 was administered orally to three groups of SD rats (n=10) at dose levels of 0, 100, 300, or 1000 mg/kg. The rats were observed at 15, 30, and 45 minutes, 1, 2, 3, 4, and 24 hours following dosing. The following parameters were recorded: seizures/convulsions, startled response, irritability, decreased grip strength, motor activity, abnormal posture, excretion, piloerection, pupil size, corneal reflex, awareness reaction, vocalization, decreased abdominal tone, body tremors, immobility, ataxia, decreased respiration, nociceptive response, and pinnal reflex. Body temperatures were recorded at 60 minutes following dose administration. Oral administration of RDX5791 at 0, 100, 300, and 1000 mg/kg did not cause any apparent neuropharmacological or toxicological signs. Body temperature was not affected by RDX5791 at 100, or 300 mg/kg. However, at 1000 mg/kg, a slight decrease in body temperature (38°C) at 60 minutes following dosing was noticed, compared to control (38.49°C).

Study: Evaluation of Respiratory Function of RDX5791 Following Single-Dose Oral Administration in Sprague Dawley Rats (Study # RDX5791-SP-03)

RDX5791 was administered orally to rats at dose levels of 0, 100, 300, and 1000 mg/kg. Respiratory parameters were recorded at 15 minutes and 1, 2, 4, and 6 hours after the administration of RDX5791. Oral administration of RDX5791 at 100 and 1000 mg/kg did not cause any statistically significant changes in respiratory rate, tidal volume or minute volume compared to predose values or to the vehicle control group at any time points. Compared to the predose values, statistically significant reduction in respiratory rate was observed at 15 minutes and 2, 4, and 6 hours following the oral administration of 300 mg/kg. Similarly, a statistically significant increase in tidal volume was observed at 2 and 6 hours at the same dose level of RDX5791 when compared with predose values. However, there were no statistically significant differences in respiratory rate or tidal volume when compared to the vehicle control group at the respective time points and the effects was not dose-dependent. Minute volume was not affected by the 300 mg/kg dose of RDX5791.

ADME/PK

Table 68. Absorption, Distribution, Metabolism, and Excretion Study Findings

Type of Study	Major Findings
Absorption	
Determination of RDX5791 plasma levels after a single oral dose in Sprague-Dawley rats (Study Number # RDX5791-PK-03 and RDX5791-PK-04)	RDX5791 was administered at a single oral dose to male Sprague-Dawley rats via oral gavage at 1, 10 or 30 mg/kg. Most of the plasma samples were found to be below the quantifiable limit (0.5 ng/mL to 2 ng/mL). The results indicate that bioavailability of RDX5791 is minimal.
Single oral dose PK study of RDX5791 in dogs (Study Number # RDX5791-PK-05)	RDX5791 was administered at a single oral dose to overnight fasted beagle dogs at dose levels of 1 or 10 mg/kg. Most of the plasma samples were found to be below the quantifiable limit of 0.5 ng/mL. As a result, PK analysis was not conducted. This indicates that RDX5791 exhibits minimal bioavailability in fasted dogs.
Single dose PK study of AZ13792925 (Study Number # RDX5791-M1-02)	AZ13792925 (major tenapanor metabolite) was administered at a single dose to C57BL/6 male mice via oral gavage at 100 or 300 mg/kg. AZ13792925 was rapidly absorbed following oral administration. T_{max} was 0.5 hour for the 100 mg/kg dose and 2 hours for the 300 mg/kg dose. C_{max} was 41,063 and 85,419 ng/mL and AUC_{0-24h} was 104,819 and 801,778 ng.h/mL for the 100 and 300 mg/kg doses, respectively. For the 100 mg/kg group, the half-life ($T_{1/2}$) was 1.29 hours. Plasma concentrations of AZ13792925 remained high 24 hours after dosing in the 300 mg/kg group (>10 µg/mL), thus a $T_{1/2}$ could not be calculated for the 300 mg/kg group.
Single and multiple dose PK of tenapanor and AZ13792925 in Sprague Dawley rats (Study Number # RDX5791-M1-05)	Male and female SD rats were administered tenapanor (5 or 10 mg/kg/day) or tenapanor metabolite (AZ13792925: 5 or 30 mg/kg/day) by oral gavage once daily for 4 consecutive days. Blood samples were collected from the animals at predetermined time points over 24 hours after the first and fourth doses. Following a single or four-consecutive oral doses of tenapanor to male and female rats at 5 or 10 mg/kg, plasma concentrations of tenapanor were below the quantification limit (BQL) (<0.500 ng/mL) in all samples. Following the first dose of 5 or 10 mg/kg tenapanor to rats, the mean C_{max} values of AZ13792925 (metabolite) for females were 7.63 and 9.05 ng/mL and in males, 2.04 and 4.13 ng/mL at the respective doses. The median T_{max} was 8.00 hours in females and 1.00 hours in males at both dose levels. The corresponding mean AUC_{last} values for AZ13792925 were 117 and 150 ng·h/mL in females and 27.0 and 45.3 ng·h/mL in males. Following a single or four consecutive oral doses of AZ13792925 to male and female rats at 5 or 30 mg/kg/day, plasma concentrations of AZ13792925 were s high in males and females. Following the first oral dose of 5 or 30 mg/kg AZ13792925 to rats, the mean C_{max} values for females in the 5 and 30 mg/kg groups were 2300 and 13200 ng/mL and in males, 1620 and 9880 ng/mL at the respective doses. The median T_{max} was 1.00 hour in females and 0.500 to 1.00 hours in males at both dose levels. The corresponding mean AUC_{last} values for AZ13792925 were 15400 and 122000 ng·h/mL in females and 9490 and 66100 ng·h/mL in males. (continued below)

Type of Study	Major Findings
Single and multiple dose PK of tenapanor and AZ13792925 in Sprague Dawley rats (Study Number # RDX5791-M1-05). (continued)	Following 4 consecutive days daily oral dosing of 5 or 30 mg/kg/day AZ13792925 to rats, the mean C_{max} values for females in the 5 and 30 mg/kg groups were 2520 and 17800 ng/mL and in males, 2550 and 12500 ng/mL at the respective doses. The corresponding mean AUC_{last} values at 5 or 30 mg/kg for AZ13792925 were 18600 and 176000 ng.h/mL in females and 11200 and 116000 ng·h/mL in males, respectively. The median T_{max} ranged from 0.500 to 1.25 hours in females and 0.250 to 0.500 hours in males at both dose levels.
Distribution	
Absorption, distribution, excretion, and PK of ^{14}C-RDX5791 following oral administration to rats (Study Number # RDX5791-PK-10).	Radiolabeled RDX5791 was administered to male SD rats at a single oral dose of 0.3 mg/kg (27.6 to 27.8 µCi/kg) or 1 mg/kg (90.7 to 94.2 µCi/kg). An oral dose of 1 mg/kg was also administered to Long Evans male rats. Following a single, 1 mg/kg oral dose to male SD rats, the mean maximum concentrations of radioactivity in the blood and plasma were 4.94 and 5.62 ng eq/g at 4 and 8 hours postdose, respectively. By 24 hours postdose (T_{last}), mean concentrations of radioactivity in blood and plasma declined to 0.477 and 0.343 ng eq/g, respectively. In both rats, the bulk of ^{14}C-RDX5791 detected in the gastrointestinal (GI) tract throughout the time course of this study. Very low levels of radioactivity were detected in urine at 2 and 4 hours postdose in Long Evans rats and at 2 through 24 hours postdose in SD rats. Tissues containing radioactivity common to both rat strains were cecum, esophagus, kidney, large intestine, liver, lungs, small intestine, spleen, and stomach. Excluding the uveal tract in the Long Evans rat, radioactivity concentrations in tissues of both rat strains were measurable only in the liver and GI tract at 24 hours postdose. For Long Evans rats, radioactivity concentrations were below measurable levels in most tissues by 72 hours postdose except for uveal tract. By 168 hours postdose, radioactivity concentrations in uveal tract declined below measurable levels. For SD male rats, radioactivity concentrations were below measurable levels in all tissues by 48 hours postdose. No metabolites were detected in feces and approximately 98% of the administered dose was unchanged ^{14}C-RDX5791, indicating that that no substantial metabolism of ^{14}C-RDX5791 occurred.
The determination of the in vitro binding of AZD1722 to plasma proteins in the mouse, rat, rabbit, dog and human (Study # RDX5791-PK-07 and ADME-AZS-Wave4-130110).	Pooled mouse, rat, rabbit, dog and human plasma samples were incubated with RDX5791 (AZD1722) at concentrations of 1µM and 100µM RDX5791 was highly bound (>99%) to mouse, rat, rabbit, dog and human plasma. The percentage of unbound drug at the concentration of 100µM RDX5791 (AZD1722) was <0.00100 for the mouse, 0.00701 for the rat, 0.00748 for the rabbit, 0.00313 for the dog, 0.00300 for the human. The percentage of RDX5791 bound to human, rat and dog plasma at 1µM concentration was 97.8, 99.9 and 100%, respectively.
The determination of the in vitro binding of AZ13792925 to plasma proteins in the mouse, rat, rabbit, dog and human (Study number # ADME-AZS-Wave4-150318).	Pooled mouse, rat, rabbit, dog and human plasma were incubated with AZ13792925 (tenapanor metabolite) at concentrations of 0.01, 0.1, 1 and 10 µmol/L. AZ13792925 was highly bound to mouse, rat, rabbit, dog and human plasma. The average percentage of unbound compound over the range 0.01 to 10 µmol/L was found to be 4.73% in the rabbit, 5.16% in the dog and 3.27% in the human. The percentage of unbound compound was concentration dependent, ranging from 2.53% at 1 µmol/L to 4.52% at 10 µmol/L for the mouse, and from 2.07% at 1 µmol/L to 2.91% at 10 µmol/L for the rat.

Type of Study	Major Findings
The determination of the in vitro blood partition of AZ13792925 in human whole blood (Study # Numbers ADME-AZS-Wave3-150331 and ADME-ADX-170704-BP)	The objective of this study was to determine the blood to plasma ratio of AZ13792925 in human whole blood. Fresh whole blood from healthy volunteers or plasma were isolated and incubated with 0.03, 0.1, 0.3, 1, 3, and 10µM of AZ13792925 (major metabolite). The blood to plasma partition ratio ($K_{b/p}$) of AZ13792925 was assessed in triplicate by measuring the concentrations in plasma and whole blood samples. The $K_{b/p}$ values at 0.03, 0.1, 0.3, 1, 3, and 10µM of AZ13792925 in human whole blood were 5.21, 5.87, 5.13, 5.33, 5.66, and 16.7, respectively, indicating AZ13792925 has the potential to distribute to blood cells.
Metabolism	
Stability of RDX5791 in simulated gastric and intestinal fluids (Study Number # RDX5791-PK-01)	Simulated gastric fluid (SGF, without pepsin) and simulated intestinal fluid (SIF, without pancreatin) were incubated with 1µM of RDX5791 containing 3 mg/mL of pepsin and pancreatin, respectively. No significant reduction in the levels of RDX5791 was observed in simulated gastric or intestinal fluids following incubation for 3-hour to 6 days.
Metabolic stability and preliminary identification of RDX5791 metabolites analysis using in vitro model of rat, dog and human metabolism (Study Number # RDX5791-PK-06).	Rat, human, or dog liver microsomes (protein concentration 0.5 mg/mL) were incubated with 1µM of RDX5791. For metabolite identification, rat, human, or dog liver microsomes (protein concentration 1 mg/mL) were incubated with 20µM RDX5791. Incubations were initiated by adding 2mM NADPH. Negative control samples were spiked with water in place of NADPH. RDX5791 was metabolized in human, rat and dog liver microsomes. The intrinsic clearance (CL_{int}) values of RDX5791 in microsomes from human, rat and dog were 1018, 298 and 947 µL/min/mg protein, respectively. RDX5791 appears to be metabolized into many minor metabolites. The most prevalent human metabolites were M1 - M5. The five most abundant human metabolites were also observed in rat and dog microsomes. No single metabolite exceeded 5% of the parent drug in any species.
Determination of the human cytochrome P450 enzymes involved in the metabolism of AZD1722 (AZ13667691) (Study Number # ADME-AZS- Wave3 [CYP isoform ID]-150527).	The aim of this study was to investigate the metabolism of AZD1722 (tenapanor) by 10 heterologously expressed human cytochrome P450 (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5). AZD1722 was incubated with P450 at concentration of 2 µmol/L for a period of 25 minutes. The concentration of CYP Protein was 100 pmol/mL. Tenapanor was metabolized to 56.8% and 39.8% by CYP3A4 and CYP3A5, respectively. CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 were weakly involved in the metabolism of tenapanor, accounting for 0.301%, 0.0450%, 0.0113%, 2.19%, 0.308%, 0.225% and 0.358%, respectively. CYP2E1 was not shown to metabolize tenapanor. CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5 were found to be involved in the formation of AZ13792925 (M1; major tenapanor metabolite). The AZ13792925 formation velocity was the highest with CYP3A4 and CYP3A5 (339 and 245 pmol/min/nmol CYP after 5 minutes of incubation, respectively).

Type of Study	Major Findings
In vitro stability of AZ13792925 in mouse, rat, and human liver microsomes (Study Number # RDX5791-M1-08)	The objective of this study was to evaluate the metabolic stability of AZ13792925 (tenapanor metabolite) in mouse, rat, and human liver microsomes. AZ13792925 was incubated at 1µM in mouse liver microsomes (MLM), rat liver microsomes (RLM), or human liver microsomes (HLM) with NADPH. Reactions were quenched at 0, 2, 4, 6, 10, 20, and 30 minutes and AZ13792925 levels were measured by HPLC-MS/MS. The mean intrinsic clearance (CL_{int}) values of AZ13792925 in MLM, RLM, and HLM were 361, 68.2, and 16.3 µL/min/mg protein. The corresponding mean half-life ($T_{1/2}$) values of AZ13792925 were 3.84, 20.4, and 91.5 minutes. The results showed that AZ13792925 was unstable in MLM and RLM, but was relatively more stable in HLM, indicating that AZ13792925 can potentially be metabolized in the liver in all three species, but may have relatively lower clearance by the human liver.
Excretion	
Quantitative determination of RDX5791 levels in a 72-hour collection of feces after a single dose in Sprague-Dawley rats (Study Number # RDX5791-PK-02)	RDX5791 was administered to male SD rats at a dose level of 0.1 mg/kg and feces were collected from 0-72 hours after dosing. The levels of RDX5791 in feces were quantitatively determined using LC-MS/MS. The mean overall recovery of RDX5791 in the 72-hour fecal sample was 92.2±1.6%.
Absorption, distribution, excretion, and PK of ^{14}C-RDX5791 following oral administration to rats (Study Number # RDX5791-PK-10)	Radiolabeled RDX5791 was administered to male SD rats at a single oral dose of 0.3 (27.6 to 27.8 µCi/kg) or 1 mg/kg (90.7 to 94.2 µCi/kg). Excretion and mass balance of the administered radioactivity were assessed by analyzing the urine and feces up to 168 hours postdose, and in bile, urine, and feces collected through 120 hours postdose in bile duct cannulated (BDC) rats. Radioactivity was eliminated predominantly via fecal excretion, with 122% and 95.3% of the administered radioactivity recovered from intact and BDC male rats, respectively. The bulk of the radioactivity was recovered within the first 24 hours after the dose administration. Urinary excretion was minimal, accounting for 0.335% and 1.78% of the total radioactivity in intact and BDC rats, respectively.
TK data from general toxicology studies A 6-month oral (Gavage) toxicity study of AZD1722 in Sprague Dawley rats with male fertility evaluation (Study # (b) (4)-775030)	Rat C_{max} *(Week 4; M/F)* 1 mg/kg/day: BQL at all time points (0.5, 1, 2, 4, 8, and 24h) for both sexes; females at 1h: 0.333 ng/mL 3 mg/kg/day: BQL at all time points for both sexes 10 mg/kg/day: Male- BQL at 0.5, 2, 4, 8, and 24h and 0.423 ng/mL at 1h; Female-BQL at 1, 2, 4, 8, and 24 h and 1.78 ng/mL at 0.5h C_{max} *(Week 26; M/F)* 1 mg/kg/day: Male-BQL at 4, 8, and 24h and 0.440, 0.860, and 0.290 ng/mL at 0.5, 1, and 2h, respectively; Female- BQL at all time points (0.5, 1, 2, 4, 8, and 24h). 3 mg/kg/day: BQL at all time points (0.5, 1, 2, 4, 8, and 24h) for both sexes 10 mg/kg/day: Male-BQL at 1h and 25.4, 0.490, 7.40, 0.850, and 0.353 ng/mL at 0.5, 2, 4, 8, and 24h, respectively; Female- BQL at all time points (0.5, 1, 2, 4, 8, and 24h).

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td>A 9-month oral (Capsule) toxicity study of AZD1722 in beagle dogs (Study # (b) (4)-775031).</td><td>Dog
C_{max} (Week 4 and Week 38; M/F)
<table>
<tr><th colspan="7">Mean AZD1722 Plasma Concentrations (ng/mL)</th></tr>
<tr><th></th><th colspan="3">Males</th><th colspan="3">Females</th></tr>
<tr><th>Dosage (mg/kg/day):</th><th>50</th><th>300</th><th>1000</th><th>50</th><th>300</th><th>1000</th></tr>
<tr><td>Study Week 4</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0.5 hours post-dosing</td><td>0.144</td><td>BLQ</td><td>BLQ</td><td>0.365</td><td>BLQ</td><td>0.715</td></tr>
<tr><td>1 hour post-dosing</td><td>0.134</td><td>0.268</td><td>1.41</td><td>0.682</td><td>0.132</td><td>1.35</td></tr>
<tr><td>2 hours post-dosing</td><td>BLQ</td><td>0.608</td><td>3.02</td><td>0.832</td><td>0.655</td><td>1.54</td></tr>
<tr><td>4 hours post-dosing</td><td>0.278</td><td>0.273</td><td>1.31</td><td>0.154</td><td>1.04</td><td>1.25</td></tr>
<tr><td>8 hours post-dosing</td><td>BLQ</td><td>0.650</td><td>1.51</td><td>BLQ</td><td>0.440</td><td>0.895</td></tr>
<tr><td>24 hours post-dosing</td><td>BLQ</td><td>0.253</td><td>0.971</td><td>BLQ</td><td>0.128</td><td>0.839</td></tr>
<tr><td>Study Week 38</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0.5 hours post-dosing</td><td>BLQ</td><td>BLQ</td><td>0.355</td><td>0.155</td><td>0.402</td><td>0.622</td></tr>
<tr><td>1 hour post-dosing</td><td>BLQ</td><td>0.388</td><td>0.886</td><td>0.452</td><td>0.943</td><td>1.13</td></tr>
<tr><td>2 hours post-dosing</td><td>0.190</td><td>0.515</td><td>2.46</td><td>0.524</td><td>1.20</td><td>2.00</td></tr>
<tr><td>4 hours post-dosing</td><td>BLQ</td><td>0.329</td><td>1.59</td><td>BLQ</td><td>0.953</td><td>0.942</td></tr>
<tr><td>8 hours post-dosing</td><td>BLQ</td><td>0.179</td><td>0.854</td><td>BLQ</td><td>0.140</td><td>0.680</td></tr>
<tr><td>24 hours post-dosing</td><td>BLQ</td><td>0.568</td><td>1.58</td><td>BLQ</td><td>0.695</td><td>0.438</td></tr>
</table>
BLQ = Below the quantifiable limit.</td></tr>
<tr><td>TK data from reproductive toxicology studies
An oral (gavage) study of the effects 0f RDX5791 on embryo/fetal development in rats (Study # (b) (4)-775024 [RDX5791-TX-14]).</td><td>Rat
C_{max} (Day 6; F)
1 mg/kg/day: BQL
10 mg/kg/day: BQL; one animal with 4.84 ng/mL at 8 hours
30 mg/kg/day: BQL; one animals at each time point of 2, 8, and 24 hours with values of 2.92, 8.17, and 0.66 ng/mL, respectively.
C_{max} (Day 17; M/F)
1 mg/kg/day: BQL; one animal with 3.75 ng/mL at 4 hours
10 mg/kg/day: BQL
30 mg/kg/day: BQL</td></tr>
<tr><td>An oral (gavage) study of the effects 0f RDX5791 on embryo/fetal development in rabbits (Study # (b) (4)-775024 [RDX5791-TX-14]).</td><td>Rabbit
C_{max} (Day 6; F)
5 mg/kg/day: BQL
15 mg/kg/day: BQL; one animal with 0.910 ng/mL at 2 hours.
45 mg/kg/day: BQL; one animal at each time point of 1, 2, 4, and 24 hours with values of 0.593, 0.545, 0.532, and 0.811ng/mL, respectively.</td></tr>
</table>

Type of Study	Major Findings
An oral (gavage) study of the effects of AZ13792925 on embryo/fetal development in rats (Study # (b) (4)-775034).	Rat T_{max}: *(Days 6 and 17; F, all doses)* 0.5 to 2 hours *AUC_{last} (Day 6; F)* 30 mg/kg/day: 237,000 ng.h/mL 150 mg/kg/day: 726,000 ng.h/mL 400 mg/kg/day: 1,150,000 ng.h/mL C_{max} *(Day 17; F)* 30 mg/kg/day: 16,700 ng/mL 150 mg/kg/day: 34,900 ng/mL 400 mg/kg/day: 55,500 ng/mL *AUC_{last} (Day 17; F)* 30 mg/kg/day: 247,000 ng.h/mL 150 mg/kg/day: 897,000 ng.h/mL 400 mg/kg/day: 1,320,000 ng.h/mL C_{max} *(Day 17; F)* 30 mg/kg/day: 17,000 ng/mL 150 mg/kg/day: 52,500 ng/mL 400 mg/kg/day: 89,200 ng/mL *Accumulation:* There was no accumulation of AZ13792925 for any group; exposure to AZ13792925 was almost similar on GDs 6 and 17 for all groups. *Dose proportionality:* Exposures of AZ13792925, in terms of AUC_{last} and C_{max}, increased with increasing dosage over the 30 to 400 mg/kg/day range. The increase in exposure to AZ13792925 was less than dose proportional in terms of AUC_{last} and C_{max} on both evaluation days.

Type of Study	Major Findings
TK data from Carcinogenicity studies A 26-week carcinogenicity study of tenapanor and AZD13792925 by oral gavage in CByB6F1/Tg RasH2 hemizygous mice (Study # 20105943 [RDX5791-TX-16)	**Day 1:** AUC_{last} (Male) of tenapanor: 10 mg/kg/day: Not calculated (NC) 30 mg/kg/day: NC 100 mg/kg/day: 11.6 ng.h/mL AUC_{last} (Female) of tenapanor: 100 mg/kg/day:105 ng.h/mL 300 mg/kg/day: 343 ng.h/mL 800 mg/kg/day: 204 ng.h/mL **Day 180:** AUC_{last} (Male) of tenapanor : 10 mg/kg/day: NC 30 mg/kg/day: 7.54 ng.h/mL 100 mg/kg/day: 21.9 ng.h/mL AUC_{last} (Female) of tenapanor : 100 mg/kg/day:11.1 ng.h/mL 300 mg/kg/day: 53.6 ng.h/mL 800 mg/kg/day: 913 ng.h/mL **Day 1:** AUC_{last} (Male) of AZ13792925 (tenapanor metabolite): 10 mg/kg/day: 1.39 ng.h/mL 30 mg/kg/day: 4.13 ng.h/mL 100 mg/kg/day: 7.00 ng.h/mL AUC_{last} (Female) of AZ13792925 (tenapanor metabolite) : 100 mg/kg/day:39.9 ng.h/mL 300 mg/kg/day: 68.7 ng.h/mL 800 mg/kg/day: 80 ng.h/mL **Day 180:** AUC_{last} (Male) of AZ13792925 (tenapanor metabolite) : 10 mg/kg/day: 2.79 ng.h/mL 30 mg/kg/day: 7.34 ng.h/mL 100 mg/kg/day: 14.8 ng.h/mL AUC_{last} (Female) of AZ13792925 (tenapanor metabolite) : 100 mg/kg/day:20.9 ng.h/mL 300 mg/kg/day: 35.5 ng.h/mL 800 mg/kg/day: 212 ng.h/mL **Day 1:** AUC_{inf} (Male) of AZ13792925 following AZ13792925 administration: 55 mg/kg/day: 16,700 ng.h/mL 165/110 mg/kg/day: 144,000 ng.h/mL AUC_{inf} (Female) of AZ13792925 following AZ13792925 administration: 55 mg/kg/day: 45,200 ng.h/mL 165/110 mg/kg/day: 290,000 ng.h/mL **Day 180:** AUC_{inf} (Male) of AZ13792925 following AZ13792925 administration: 55 mg/kg/day: 31,200 ng.h/mL 165/110 mg/kg/day: 89,500 ng.h/mL AUC_{inf} (Female) of AZ13792925 following AZ13792925 administration: 55 mg/kg/day: 59,100 ng.h/mL 165/110 mg/kg/day: 197,000 ng.h/mL

Abbreviations: AUC, area under the curve; BDC, bile duct cannulated; BQL, below quantification limit; C_{max}, maximum concentration; GI, gastrointestinal; HLM, human liver microsome; HPLC-MS/MS, high performance liquid chromatography coupled with tandem mass spectrometry; $K_{b/p}$, blood to plasma partition ratio; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MLM, mouse liver microsome; PK, pharmacokinetics; RLM, rat liver microsome; SD, Sprague Dawley; TK, toxicokinetic

Toxicology

Study Title/Number: A 28-Day Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study of AZ13792925 in Hemizygous RasH2 [CbyB6F1-Tg(HRAS)2Jic] Mice (Study # (b) (4) -775038)

Key Study Findings

- Once-daily oral administration of AZ13792925 (major metabolite of tenapanor) at dosage levels of 75, 150, and 250 mg/kg/day or tenapanor at a dosage level of 400 mg/kg/day to male and female Tg rasH2 mice for 28 consecutive days was well tolerated at all dosages, and all animals survived to scheduled necropsy.
- There were no test article-related clinical observations, changes in body weights, food consumption, hematology, serum chemistry, organ weight values, or gross or microscopic pathology.
- The NOAEL was determined to be 250 mg/kg/day for AZ13792925.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Study: A 28-Day Oral (Gavage) Toxicity and Toxicokinetic Study of RDX5791 With a 14-Day Recovery Period in Sprague Dawley Rats (Study # (b) (4) -775003 – RDX5791-TX-05)

RDX5791 was administered orally once daily to Sprague-Dawley rats for a minimum of 14 consecutive days at dose levels of 0, 30, 100, 300, and 1000 mg/kg/day. Due to high mortality and adverse effects on body weight, this study was shortened from 4 weeks to 2 weeks. Mortality was observed in all dose groups. One female, three females, three animals (one male and two females) and four females died from 30, 100, 300 and 1000 mg/kg/day dose groups, respectively as early as study Day 12. All other animals survived up to the end of the study. All survived animals exhibited soft feces and diarrhea with brown material around the anogenital area throughout the dosing and recovery periods. Both male and female rats at all doses showed dramatic reduction of body weight from 26% to 42% during the dosing period. However, during the recovery period, there was an increase in body weight gain, but it remained lower than the control group. Test article-related lower food consumption and higher water consumption were noted during the dosing period in both males and females at all doses. Hematology assessments showed:

- Higher red blood cell count (up to 12% in males; up to 23% in females)
- Higher hemoglobin (up to 14% in males; up to 21% in females)
- Higher hematocrit (up to 11% in males and up to 16% in females)
- Higher mean corpuscular hemoglobin concentration
- Higher red blood cell distribution width
- Higher hemoglobin distribution width in both males and females
- Higher neutrophil count (up to 221%), and monocyte count (up to 113%)
- Lower mean corpuscular volume (up to 5.9%) in females only

However, the changes were generally not dose-dependent. Test article-related serum chemistry changes included:

- Lower sodium (up to 8% in males; up to 13% in females)
- Lower potassium (up to 16.4% in males; up to 3.0% in females)
- Lower albumin (up to 9% in males and females)
- Lower cholesterol (up to 6.0% in males; up to 35% in females)
- Higher urea (up to 131% in males; up to 134% in females)
- Higher alanine aminotransferase (up to 100% in males; up to 59% in females)
- Higher aspartate aminotransferase (up to69% in males; up to 21% in females)
- Higher creatinine (males: 50% at high dose; females: 50% at high dose)

Gross pathology examinations showed distended and/or brown fluid contents in the cecum, colon, duodenum, ileum, jejunum, and stomach at the time of primary necropsy or in animals found dead. Test article-related histopathologic findings were noted in the cecum, colon, duodenum, ileum, jejunum and rectum of both males and females in all dose groups. These organs showed reduced goblet cells accompanied by mixed inflammatory infiltration in the cecum and rectum and mucosal erosion and necrosis of glandular epithelial cells of the rectum. The kidney of males and females showed multifocal hyaline casts in association with tubular degeneration and regeneration. Mesenteric lymph nodes, spleen, thymus, and femoral and sternal bone marrow of males and females showed lymphoid depletion of variable severity. Findings in the cecum, colon, duodenum, ileum, jejunum, rectum, and kidneys recovered in the 30 mg/kg/day group after a 14-day recovery period.

Study: A 3-Month Oral Gavage Toxicity and Toxicokinetic Study of RDX5791 With a 28-Day Recovery Period in Sprague Dawley Rats (Study # (b) (4)-775022 -RDX5791-TX-10)

RDX5791 was administered orally once daily to Sprague-Dawley rats for 91 consecutive days at dose levels of 0 (0.1% Tween 80 in deionized water), 0.1, 1.0, and 5.0 mg/kg/day. There were no test article-related effects on survival, hematology, ophthalmic, or macroscopic parameters. There were no toxicological relevant differences in serum chemistry or urinalysis parameters and no adverse differences in body weights, food consumption values, organ weights, and microscopic findings. The most prominent test article-related clinical observations consisted of soft feces, which was consistent with the pharmacology of the test article. Full recovery from these findings occurred during the recovery period. The NOAEL was determined to be 5 mg/kg/day.

Study: A 3-Month Oral (Capsule) Toxicity Study of RDX5791 in Beagle Dogs With a 28-Day Recovery Period (b) (4)-775023-RDX5791-TX-11)

RDX5791 was administered orally to beagle dogs as capsules once daily for 91 consecutive days at dose levels of 50, 300 and 1000 mg/kg/day. All animals survived to the scheduled necropsies. Abnormal excreta (diarrhea, diarrhea containing red material, feces containing red material, mucoid feces, red mucoid feces, yellow mucoid feces, and/or soft feces), were noted across all dosage levels for most of the dosing period and sporadically throughout the recovery period. These observations were expected pharmacological effects of the test article. Body weights, body weight changes, food consumption, clinical pathology, urinalysis, ECG parameters,

ophthalmic examinations, and organ weights were unaffected by test article administration. There were no macroscopic or microscopic findings indicative of a test article-related effect. The NOAEL was determined to be 1000 mg/kg/day.

Study: A 14-Day Repeat Dose Toxicity Study of AZ13792925 in Mice (RDX5791-M1-04)

AZ13792925 (major tenapanor metabolite) was administered by oral gavage to six male and six female CByB6Fl/J mice for 14 consecutive days at dose levels of 0, 500 or 1000 mg/kg/day. All six males and six females dosed with 1000 mg/kg/day and three of six males and three of six females dosed with 500 mg/kg/day AZ13792925, were found dead or euthanized in extremis during the course of the study between Days 3 and 9. Clinical observations prior to deaths included lethargy, labored breathing, shaking, drooling, hunched, cold to touch, piloerection and moribund. High plasma exposures were observed in surviving mice, approximately 53 to 54 µg/mL and 2 to 5 µg/mL at 30 minutes and 24 hours, respectively, after the final dose. Repeat oral administration of AZ13792925 at 500 and 1000 mg/kg/day was not tolerated in male and female CByB6Fl/J mice.

Genetic toxicology

16.2.1. In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study Title/Number: Bacterial Reverse Mutation Assay (Study # RDX5791-GT-02 [AD07FF.503.BTL])

Key Study Findings:

- RDX5791 had no mutagenic potential in the bacterial reverse mutation assay.

GLP compliance: Yes

Test system: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, *E Coli* WP2 *uvrA;* dose levels of RDX5791 were 1.5, 5, 15, 50, 150, 500, 1500 or 5000 µg/plate; in the presence or absence of S9 mix.

Study is valid: Yes

16.2.2. In Vitro Assays in Mammalian Cells

Study Title/Number: In vitro Mammalian Chromosome Aberration Test (Study # RDX5791-GT-03 [ADO7FF.341.BTL])

Key Study Findings:

- RDX5791 was negative for the induction of structural and numerical chromosome aberrations in both nonactivated and S9-activated test systems in the in vitro mammalian chromosome aberration test using human peripheral blood lymphocytes.

GLP compliance: Yes

Test system: Human peripheral blood lymphocytes treated with RDX5791 at dose levels of 0, 62.5, 125, 250, 500, 1000, 2000, 2500 and 3000 µg/mL in the presence or absence of S9 mix. For positive controls, Cyclophosphamide (10 and 15 µg/mL) and Mitomycin C (0.3 and 0.6 µg/mL) were used.

Study is valid: Yes

16.2.3. In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study Title/Number: In Vivo Micronucleus Assay in Mice (Study # RDX5791-GT-04 [AD60MY.123021.BLT])

Key Study Findings:

- RDX5791 (tenapanor) was negative in the in vivo micronucleus assay in female mice at oral doses up to 2000 mg/kg.

GLP compliance: Yes
Test system: RDX5791 was administered orally to ICR mice at doses of 0, 500, 1000 and 2000 mg/kg. Animals were sacrificed approximately 24 hours after administration of the test article and bone marrow was harvested from the femur. Bone marrow smears were prepared on glass slides and stained. The stained slides were coded and 2000 polychromatic erythrocytes, including micronucleated polychromatic erythrocytes, were counted for each animal for the presence of micronuclei and the group frequencies were statistically analyzed.
Study is valid: Yes

Study Title/Number: AZD1722: Genetic Toxicity Evaluation Using the Rat Micronucleus Test (Study # 3585QR-AZD1722)

Key Study Findings:

- AZD1722 (tenapanor) was negative in the in vivo micronucleus assay in male Wister Han rats following intravenous doses up to 10 mg/kg.

GLP compliance: Yes

Test system: AZD1722 (tenapanor) was administered as two IV doses to male Wister Han rats at doses of 0, 1, 5, and 10 mg/kg 24 hours apart. Animals were sacrificed approximately 24 hours after the second dose and the bone marrow was harvested from the femur. Bone marrow smears were prepared on glass slides and stained. The stained slides were coded and 2000 polychromatic erythrocytes, including micronucleated polychromatic erythrocytes, were counted for each animal for the presence of micronuclei and the group frequencies were statistically analyzed.

Study is valid: Yes

16.2.4. Other Genetic Toxicity Studies

Study Title/Number: AZ13792925: Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in *Salmonella typhimurium* TA1535, TA100, TA1537 and TA98 and *Escherichia coli* WP2 *uvrA* (pKM101) (Study # 3581BV [8321467])

Key Study Findings:

- AZ13792925 (major tenapanor metabolite) had no mutagenic potential in the bacterial reverse mutation assay.

GLP compliance: Yes

Test system: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E Coli* WP2 *uvrA;* dose levels of AZ13792925 were 5, 16, 50, 160, 500, 1600 or 5000 µg/plate, in the presence or absence of S9 mix.

Study is valid: Yes

Study Title/Number: AZ13792925: In vitro Micronucleus Assay Using L5178Y Cells (Study # 8321468 [3522QV])

Key Study Findings:

- AZ13792925 was negative for the induction of micronuclei in L5178Y mouse lymphoma cells in the presence or absence of S-9 mix.

GLP compliance: Yes

Test system: L5178Y mouse lymphoma cells treated with AZ13792925 (metabolite of tenapanor) at dose levels of 0, 15, 30, 45, 60, 70, 80, 90, 95, 100, 105, 110, 120 and 130 µg/mL in the presence or absence of S9 mix. For positive controls, noscapine (35, 40 and 45 µg/mL), mitomycin C (0.15 and 0.30 µg/mL) and cyclophosphamide (4 and 6 µg/mL) were used.

Study is valid: Yes

16.2.5. Fertility and Early Embryonic Development Studies

Study Title/Number: AZD1722: Oral Fertility and Early Embryonic Development Study in the Female Mouse (Study # 496575 [0967GM])

Key Study Findings

- Female fertility, mating or pregnancy performance was not affected by oral administration of tenapanor to female CD-1 mice at doses up to 50 mg/kg/day.
- The NOAEL for female fertility was determined to be 50 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 69: Methods of Study 496575 in the Female Mouse

Parameter	Details
Dose and frequency of dosing:	0, 10 and 50 mg/kg/day. AZD1722 was administered daily for 14 days before pairing and continued for 6 days after mating.
Route of administration:	Oral gavage
Formulation/Vehicle:	0.1% Polysorbate 80 in Milli-Q water
Species/Strain:	Crl:CD-1 Mouse
Number/Sex/Group:	20 females/dose group
Satellite groups:	None
Study design:	AZD1722 (tenapanor) was administered orally to 10-11 weeks old CD-1 female mice once a day for 14 days prior to pairing (pairing Day 15) then continuing through pairing until Day 6 of gestation. Males were not dosed with the test article. Control animals received the vehicle only. Each female was paired with its designated male for a maximum of 12 nights. At the end of the pairing period, if no evidence of mating was observed, the female was returned to an individual cage. Mortality/moribundity, clinical signs, body weights and food consumption were recorded. Vaginal lavages were taken early each morning, commencing on the day of pairing, until a positive mating sign had occurred, and the stage of estrus observed in each lavage was recorded. The females were sacrificed on Day 12 or 13 of gestation, the ovaries weighed, and pregnancy parameters were assessed.
Deviation from study protocol affecting interpretation of results:	None

Table 70: Observations and Results of Study 496575 in the Female Mouse

Parameters	Major Findings
Mortality	None.
Clinical Signs	No treatment-related clinical signs were observed.
Body Weights	Slight transient statistically significant increases in body weight gain were noted in the 50 mg/kg/day AZD1722 dosed animals compared to controls. These transient increases resulted in a total mean body weight gain of 0.9 g over the 14-day pre-pairing period for the high dose group (50 mg/kg/day) compared to 0.6 g and 0.7 g, respectively, for the low dose (10 mg/kg/day) and control group animals. These increases in body weight gain at 50 mg/kg/day were minor and not considered to be test article-related.
Necropsy findings *[Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.]*	AZD 1722 (tenapanor) had no effects on the following pregnancy data. Mating/fertility index (%) CG: 88 LD: 96 HD: 100 Copulation index male (%) CG: 88 LD: 96 HD: 100 Implantations, number CG: 286 LD: 314 HD: 334 Early embryonic deaths (%) CG: 4 LD: 7 HD: 4

Abbreviations: CG, control group; LD, low dose; MD, mid dose; HD, high dose

Study Title/Number: A 6-Month Oral (Gavage) Toxicity Study of AZD1722 in Sprague Dawley Rats With Male Fertility Evaluation (Study # (b) (4) -775030)

Key Study Findings

- Oral administration of AZD1722 (tenapanor) to SD rats at doses up to 10 mg/kg/day had no effect on male reproductive performance or precoital intervals.
- Intrauterine survival of the embryos (pre- and postimplantation losses and numbers of corpora lutea, implantation sites, and viable embryos) was unaffected by test article administration to male rats at dosage levels of 1, 3, and 10 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 71: Methods of Fertility and Early Embryonic Development Study in the Rat

Parameter	Details
Dose and frequency of dosing:	0, 1, 3, and 10 mg/kg/day. AZD1722 (tenapanor) was administered daily for approximately 10 weeks, and paired with naïve females for 13 days.
Route of administration:	Oral gavage
Formulation/Vehicle:	0.1% Tween 80 [w/v] in deionized water
Species/Strain:	Sprague Dawley rat
Number/Sex/Group:	20 males/dose group
Satellite groups:	None
Study design:	AZD1722 was administered orally to male Sprague Dawley rats once a day for approximately 10 weeks at dose levels of 0, 1, 3, and 10 mg/kg/day before pairing. Males were paired with naïve females for a period of up to 13 days. The naïve females were not dosed but were used for male fertility assessments. Control animals received the vehicle only. The day when evidence of mating was identified was termed GD 0. Following 105 days of dose administration, five males/toxicology group were euthanized and discarded without examination; the remaining 15 rats/sex/group were euthanized following 182-183 days of dose administration. Clinical signs, body weights and food consumption were recorded during the study. A laparohysterectomy was performed on GD 15 for each female with evidence of mating; laparohysterectomies included a gross necropsy focusing on reproductive tissues, examination of the uterus, confirmation of mating, and recording the number and location of corpora lutea, embryos, early resorptions, and implantation sites.
Deviation from study protocol affecting interpretation of results:	None

Abbreviations: GD, gestation day

Table 72: Observations and Results of Fertility and Early Embryonic Development Study in the Rat

Parameters	Major findings
Mortality	Two males (#9102 and #9106) from the 10 mg/kg/day group died on Days 65 and 139, respectively, after dosing. All females with evidence of mating survived to the scheduled laparohysterectomy (GD 15).
Clinical signs	Soft feces, diarrhea, and associated brown material around the anogenital and urogenital areas were noted in a dose-related manner in the males receiving 1, 3, and 10 mg/kg/day doses. These observations were the expected pharmacological effects of the test article.
Body weights	Mean maternal body weights and body weight gains were similar among all groups of untreated females throughout gestation.
Necropsy findings [mating/fertility index, corpora lutea, preimplantation loss, etc.]	No test article-related effects on male reproductive performance (mating, fertility, and copulation indices) or on the mean number of days between pairing and coitus were observed at any dosage level. Mating/fertility index (%) CG: 100 LD: 100 MD: 100 HD: 100 Copulation index male (%) CG:100 LD: 100 MD: 100 HD: 94.7 Fertility index, Female (%) CG: 100 LD: 100 MD: 100 HD: 100 Corpora lutea, number CG: 324±1.8 LD: 327±2.6 MD: 344±2.09 HD: 290±1.5 Implantation site, (%), CG: 15.2 LD: 15.4 MD: 16.2 HD: 15.8 Pre-implantation lost (%) CG: 7.5 LD: 5.3 MD: 5.7 HD: 2.0; lower than control group but not statistically significant. Live embryos, number CG: 289±3.25 LD: 287±2.5 MD: 298±2.2 HD: 270±1.9 Postimplantation loss (%) CG: 4.5 LD: 7.0 MD: 7.7 HD: 5.0

Abbreviations: CG, control group; GD, gestation day; HD, high dose; LD, low dose; MD, middle dose

16.2.6. Embryo-Fetal Development Studies

Study Title/Number: An Oral (Gavage) Study of the Effects of AZ13792925 on Embryo-Fetal Development in Rat (Study # (b) (4) -775034)

Key Study Findings

- AZ13792925 (major tenapanor metabolite) was administered to pregnant females once daily by oral gavage from Gestation Day 6 to Gestation Day 17 at dose levels of 0, 30, 150, and 400 mg/kg/day. Females in the HD group showed moribundity, adverse clinical observations, and reduced body weight and food consumption.
- A higher mean litter proportion of postimplantation loss with a corresponding reduction in viable fetuses, lower mean fetal body weights, higher incidences of fetal malformations (edema, fetal anasarca, and costal cartilage anomaly) and developmental variations (pale spleen, small spleen, 7th cervical rib(s), 14th rudimentary rib(s), slightly or moderately misaligned sternebra(e), and ossification-related skeletal variations) were noted at 400 mg/kg/day. These findings at the 400 mg/kg/day dose are likely to be related to maternotoxicity (moribundity, abnormal clinical signs, and decreased body weight gain).
- The NOAEL for maternal toxicity and embryo-fetal development was determined to be 150 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 73: Methods of Study (b) (4) -775034 on Embryo-Fetal Development in Rat

Parameter	Details
Dose and frequency of dosing:	0, 30, 150 and 400 mg/kg/day, once daily
Route of administration:	Oral gavage
Formulation/Vehicle:	0.1% Tween 80 [w/v] in deionized water
Species/Strain:	Crl:CD (Sprague Dawley) rats
Number/Sex/Group:	25 females/dose group
Satellite groups:	Yes, eight females/dose group for TK analysis. Blood samples were collected from four rats/group at predose and approximately 0.5, 1, 2, 8, and 24 hours after dose administration on GDs 6 and 17.
Study design:	Pregnant females were dosed once daily by oral gavage, from GD 6 to GD 17. Females were observed daily, and their body weights recorded; food intake was monitored daily. On GD 20, a laparohysterectomy was performed on each surviving female, and the uteri, placentae, and ovaries were examined, and the number of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weight and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations.
Deviation from study protocol affecting interpretation of results:	None.

Abbreviation: GD, gestation day; TK, toxicokinetic

Table 74: Observations and Results of Study (b) (4)-775034 on Embryo-Fetal Development Study in the Rat

Parameters	Major findings
Mortality	One female (#6272) in the 400 mg/kg/day group was euthanized *in extremis* on GD 17 following marked body weight losses and reduced food consumption during GDs 6 to 16, and pale body and gasping on the day of euthanasia.
Clinical Signs	In the 400 mg/kg/day dose group, decreased defecation, pale feces, red vaginal discharge, rales, pale extremities, and red material on various body surfaces (nose, forelimbs, and mouth) were noted for the surviving females. These observations were generally noted throughout the treatment period, beginning as early as GD 6. In the 150 mg/kg/day group, rales were noted. In the absence of other overt signs of toxicity at this dosage level, this finding was considered test article-related but not adverse. No other test article-related clinical signs were noticed.
Body Weights	There was a reduction in overall mean body weight gain of 19% in HD during the treatment period (Days 7 to 18 of gestation) compared with controls; mean body weight gain was reduced by 4.5% to 5.5% at MD during GDs 7 to 9 compared to controls. Mean body weights in this group were similar to the control group during GDs 10 to 20. Mean body weight in the LD group were unaffected by the test article administration.
Necropsy findings Cesarean section data	Corpora lutea, number CG: 428±2.7 LD: 406±1.7 MD: 419±1.4 HD: 410±2.0 Implantations, number CG: 409±1.8 LD: 395±1.6 MD: 404±2.0 HD: 400±1.8 Early Resorption (%) CG: 3.3 LD: 6.0 MD: 4.8 HD: 30.8* (significantly different from the control, p=0.01) Late Resorption (%) CG: 0.2 LD: 0.0 MD: 0.2 HD: 9.3* (significantly different from the control, p=0.05) Pre-implantation loss (%) CG: 3.7 LD: 2.6 MD: 3.8 HD: 2.3 Post implantation loss (%) CG: 3.5 LD: 6.0 MD: 5.0 HD: 40.1* (significantly different from the control, p=0.01) Fetal Weight (g) CG: 3.7 LD: 3.7 MD: 3.7 HD: 2.3* (significantly different from the control, p=0.01)

Parameters	Major findings
Necropsy findings Offspring malformations, variations, etc.	Treatment- related malformations were observed in 0(0), 0(0), 4(3), and 22(7) fetuses /litters in the control, 30, 150 and 400 mg/kg/day groups, respectively. They are: CG: No malformation LD: No malformation MD: Pale spleen; slightly higher incidences of the skeletal variations 7th cervical rib(s) and sternebra(e) nos. 5 and/or 6 unossified HD: Localized fetal edema and fetal anasarca; pale spleen; costal cartilage anomaly; higher incidences of 7th cervical rib(s) and sternebra(e) nos. 5 and/or 6 unossified and a lower incidence of cervical centrum no. 1 ossified, and higher incidences of reduced ossification of the vertebral arches and the 13th ribs(s), 14th rudimentary rib(s), sternebra(e) nos. 1, 2, 3, and/or 4 unossified; reduced ossification of the skull, pubis unossified, and slight or moderate misaligned sternebra(e).

Abbreviations: CG, control group; LD, low dose; MD, mid dose; HD, high dose; GD, gestation day

The findings at the high dose of AZ13792925 are likely to be related to maternotoxicity (moribundity, abnormal clinical signs, decreased body weight gain).

16.2.7. Juvenile Toxicology Studies

Study: A Pilot 21-Day Repeat Dose Toxicity Study of Tenapanor in Male and Female Juvenile Rats (Post Natal Days 5 to 26) (Study # 1884TOX)

In a dose ranging study in rats, tenapanor was administered orally at dose levels of 0, 5, and 10 mg/kg/day to male and female SD rats beginning at Postnatal Day (PND) 5. Tenapanor was not tolerated in female rats at either doses and in males at 10 mg/kg leading to unscheduled termination of the study on Day 11 (PND 16) due decreased body weight gain (>20%) compared to vehicle control.

Study: An Oral (Gavage) Dose Range-Finding Study of Tenapanor in Juvenile Rats (Study # (b) (4) -775062)

Tenapanor was administered to juvenile Crl:CD (Sprague Dawley) rats from PND 5 through PND 24 at doses of 0, 0.1, 0.5, 2.5 or 5 mg/kg/day once daily by oral gavage. One, four, and five males and one, four, and five females from the toxicokinetic groups died in the 0.5, 2.5, and 5 mg/kg/day groups, respectively. In addition, three, six, and seven males and two, nine, and eight females in these same respective groups of the main study were found dead or euthanized *in extremis*. These unscheduled deaths were noted as early as PND 8. Majority of them occurred during PND 15 to 25. Clinical observations of dermal atonia and unkempt appearance were noted for a majority of these animals at daily examinations beginning up to 8 days prior to death/euthanasia. Lower mean body weight gains or mean body weight losses were generally noted in the 5 mg/kg/day group males and females beginning at the start of dosing and continuing through PND 23 (males) or 22 (females). Mean body weights were 47.3% lower for males on PND 23 and 35.2% lower for females on PND 22, when compared to the control group. Slightly lower mean tibial lengths (4.9% to 11.4%) were noted in the 0.5, 2.5, and 5 mg/kg/day group males and females on PND 25. Tibial length reductions were correlated with reduced body weight in these groups. Lower spleen, thymus, and/or ovarian weights were

noted at 0.1, 0.5, 2.5, and 5 mg/kg/day dose groups. Test article-related gastrointestinal distension and microscopic bone findings of increased osteoclasts, eroded bone and/or decreased bone in sternum and/or femorotibial joint were noted in the 0.5, 2.5, and 5 mg/kg/day group males and females. The NOAEL was not determined in this study due to toxicity at all doses.

An 8-week Oral (Gavage) Toxicity Study of Tenapanor in Juvenile Rats With a 4-Week Recovery Period (Study # 00775063)

In an 8-week juvenile toxicology study followed by a 4-week recovery period, tenapanor was administered to male and female rats orally beginning at PND 5 at dose levels of 0, 0.3, 0.1, and 0.3 mg/kg/day. Animals were evaluated for clinical signs, body weights, food consumption, tibial lengths, ophthalmology, toxicokinetic parameters, clinical pathology, gross necropsy findings, organ weights, neuropathology examinations, and histopathologic examinations. Animals were also examined for developmental and neurobehavioral evaluations, estrous cycles, reproductive performance, parturition, litter viability and survival.

In the high dose group (0.3 mg/kg/day), five males (animal numbers 3561-05, 3562-04, 3591-06, 3598-04 and 3603-06) and one female (3600-13) were found dead and one female (3587-07) was euthanized *in extremis* during the dosing period. Males were found dead on PND 11, 18, 19 and 45, one female died on PND 17, and another female was euthanized on PND 24. The deaths and morbidity in the 0.3 mg/kg/day group were considered test article-related. In the 0.1 mg/kg/day dose group, one male (3626-06) and one female (3574-10) died on PND 57 and 58, respectively. In the 0.1 mg/kg/day dose group, no noteworthy changes in body weight, macroscopic, or microscopic findings were noted in the male, and the cause of death could not be determined. One female in the control group was found dead on PND 10, but the cause of death was not known.

Clinical observations revealed an incidence of brown material around the anogenital area and increased incidence of unkempt appearance was noted in the 0.3 mg/kg/day group males and females. Other clinical observation findings noted in the test article-treated groups, include red material around the nose, soft and/or mucoid feces, and/or yellow material around the urogenital or anogenital area. In the 0.3 mg/kg/day dose group, mean absolute body weights were lower up to 15.8% and 16.8% in males and females, respectively, compared to the control group during the dosing period. During the recovery period, mean body weights and body weight gains in the 0.3 mg/kg/day group males and females were comparable to the control group. Mean tibial lengths in the 0.3 mg/kg/day group were comparable to the control group during PND 5 to 19 (males) or PND 5 to 15 (females). Whereas, the mean tibial lengths from PND 22 (males) or 19 (females) were generally shorter than the control group for the remainder of the dosing period, and differences were generally statistically significant. No test article-related effects on mean body weights and body weight gains were noted in the 0.03 and 0.1 mg/kg/day dose groups. Hematology, serum chemistry, and urinalysis parameters were unaffected by test article administration. There were no test article-related macroscopic, microscopic, or organ weight changes noted.

There were no test article-related effects on the mean body weight at the attainment of balanopreputial separation and vaginal patency at any dosage level. No test article-related effects on reproductive performance (male and female mating and fertility, male copulation, and female conception indices), mean estrous cycle length, gestation length, or the process of parturition were noted at any dosage level. The mean numbers of implantation sites in the treated groups were comparable to the control group. Mean F_1 number of pups born, live litter size on PND 0, the percentage of males at birth, postnatal survival, clinical condition of pups, and mean pup body weights and body weight gains were unaffected by parental test article administration.

Based on test article-related mortality/moribundity at 0.3 mg/kg/day, the NOAEL was determined to be 0.1 mg/kg/day.

16.3. OCP Appendices (Technical Documents Supporting OCP Recommendations)

16.3.1. Clinical Pharmacology Studies

Clinical Pharmacology studies supporting the tenapanor development program are summarized in Table 75. Among these studies, the two in vivo DDI studies, i.e., TEN-01-103 and TEN-01-104, were conducted using the final, to-be-marketed (TBM) formulation. Of note, the Applicant has not demonstrated the comparability between the final TBM formulation and the formulations used in other phase 1 and phase 2 clinical studies.

Table 75. Summary of Clinical Pharmacology Studies Supporting the NDA

Study	Formulation	Dose	Clinical Pharmacology Data/Information
D5611C00007 (mass balance study)	Solution	Single dose of ^{14}C tenapanor HCl 15 mg	ADME
TEN-01-103 (DDI with cefadroxil/ midazolam)	Tablet*	50 mg BID for 12 or 13 days	PK: cefadroxil; midazolam, 1-OH-midazolam, and 4-OH-midazolam; tenapanor and M1
TEN-01-104 (DDI with itraconazole)	Tablet*	50 mg single dose	PK: tenapanor, M1
D5611C00005 (Ethnic difference)	Capsule	180 mg single dose; 15 mg (14 mg) QD to 90 mg (88 mg) BID for 7 days	PK: tenapanor and M1; PD: QT evaluation, fecal sodium excretion
D5611C00003, Part A (food effect on PD)	Free base tablet	14 mg BID for 4 days	PK: tenapanor, all BLQ; PD: fecal sodium excretion
RDX5791-201 (Phase 2a trial in IBS-C)	Capsule	10, 30, 100 mg QD for 4 weeks	Dose-response (change in CSBM frequency)
D5612C00001 (Phase 2b trial in IBS-C)	Tablet	5, 20, 50 mg BID for 12 weeks	PK: tenapanor and M1
D5611C00001 (ESRD-HD patients)	[redacted] (b) (4)		PK: tenapanor (all BLQ) and M1
RDX5791-101 (PK-PD study)	Capsule	10 mg (9.3 mg) to 900 mg (840 mg) single dose; 3 mg (2.8 mg) to 100 mg (93 mg) QD for 7 days	PK: tenapanor, all BLQ; PD: fecal sodium excretion
RDX5791-102 (PD study)	Capsule	30 mg (28 mg) QD to 30 mg (28 mg) TID for 7 days	PD: fecal sodium excretion
D5611C00002 (formulation effect on PD)	Capsule, tablet, free base tablet	14 mg BID for 4 days	PK: tenapanor, all BLQ; PD: fecal sodium excretion
D5611C00003*, Part B (Gastric pH effect on PD)	Free base tablet	56 mg BID for 4 days	PK: tenapanor, all BLQ; PD: fecal sodium excretion
D5613C00003 (DDI with midazolam)	Tablet	15 mg (14 mg) BID for 14 days	PK: tenapanor and M1
D5613C00004 (DDI with cefadroxil)	Tablet	15 mg (14 mg) BID for 4 days	PK: tenapanor, all BLQ
D5611C00006 (DDI with sevelamer carbonate)	Tablet	15 mg (14 mg) BID for 4 days	PK: tenapanor, all BLQ; PD: fecal sodium excretion
D5610C00001 (CKD patients)	Capsule	5, 15, 30, 60 mg BID for 12 weeks	PD: fecal sodium excretion

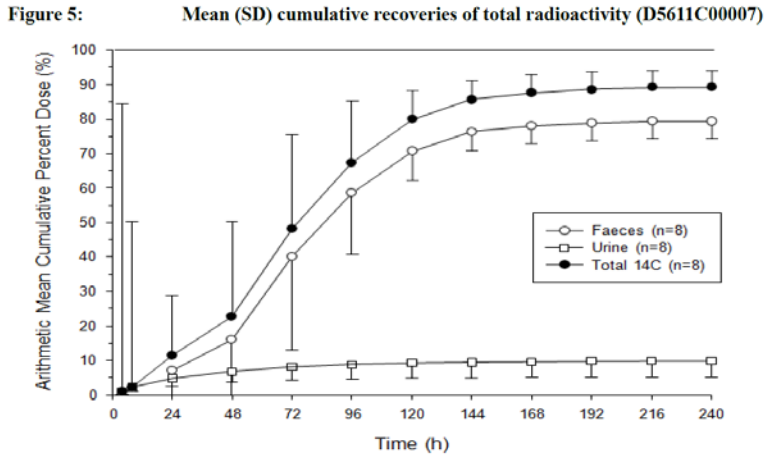
* Studies TEN-01-103 and TEN-01-104 were conducted using the final, to-be-marketed formulation.

1. D5611C00007: mass balance study to characterize the absorption, distribution, metabolism and elimination of tenapanor

Following oral administration of a single 15 mg radiolabeled ^{14}C-tenapanor dose to 8 healthy volunteers, the mean (SD) cumulative recoveries of total radioactivity in feces and urine are shown in Figure 8. The results showed that 89.2% of the total radioactivity was recovered in

urine and feces combined (79.3% in feces and 9% in urine). The recovery result in urine demonstrated that at least 9% of the total dose of ^{14}C-labelled tenapanor was absorbed into systemic circulation.

Figure 8. Mean (SD) Cumulative Recoveries of Total Radioactivity in Faeces and Urine in Study D5611C00007



Source: Study Report D5611C00007, Figure 3.

The species of parent drug and metabolites were assessed in pooled urine and feces samples. In pooled urine collected through 144 hours after dosing, approximately 8.67% of administered radioactivity was recovered in urine, of which M1 was the most abundant metabolite representing 1.49% of the dose whereas parent tenapanor was not detected (Table 76). In pooled feces collected through 144 hours after dosing, approximately 76.2% of the administered dose was excreted in feces, of which unchanged tenapanor accounted for 65.3% of the administered dose (Table 76).

Table 76. Quantitative Estimates of Tenapanor and Metabolites in Excreta and Plasma Collected after a Single Oral Administration of Tenapanor in Study D5611C00007

Compound	LC-RAM Retention t_R (min)	$Urine_{0-144h}$ (% Dose)	$Feces_{0-144h}$ (% Dose)	$Plasma_{0-24h}$ (% AUC_{0-24h})
M11	25.0	0.62	ND	5.0
M12	26.8	0.55	ND*	5.1
M13	32.8	0.32	ND	ND
M14	33.6	0.70	ND	ND
M1(NTX0004411)	34.9	1.49	ND*	16.0
M15	35.5	0.55	ND	4.1
M7	38.1	0.60	ND*	ND
M16	42.6	ND	2.4	ND
M2	44.6	ND	ND	7.6
Tenapanor	53.6	ND	65.3	ND
Sum [a]		4.8	67.7	37.8
Total [b]		8.67	76.2	

[a] Sum of quantified metabolites (≥ 3% of total integrated radioactivity in the analyzed sample) expressed as percent of administered dose in excreta and percent of AUC_{0-24h} in plasma.

[b] Total radioactivity in samples expressed as percent of administered dose in excreta as derived from study D5611C00007.

ND Not detected by RAM and MS.

* MS peak was detected but the signal was too weak to confirm the presence of the corresponding metabolite.

LC-RAM = with liquid chromatography combined with radioactivity monitoring

ND = not detected by RAM and MS

Source: Metabolite profiling report of D5611C00007 (Report BS000381-38), Table 2

In blood samples collected up to 120 hours after dosing, tenapanor were not quantifiable (< 0.5 ng/mL), suggesting that tenapanor undergoes extensive first pass metabolism following absorption. PK parameters of tenapanor based on radioactivity were obtained in plasma and whole blood. The median T_{max} of radioactivity in plasma and whole blood were 2 and 4 hours after dosing, respectively. Observed radioactivity was 5-fold greater in whole blood compared to plasma based on mean AUC_{0-t}. Radioactivity was eliminated with half-lives of 35.7 and 70.7 hours in plasma and whole blood, respectively. The mean apparent clearance (CL/F) was 11.7 L/hr. The mean renal clearance was 1.15 L/hr in plasma. In plasma collected up to 24 hours, unchanged tenapanor was not detected and M1 was the most abundant metabolite (16% of AUC_{0-24h} in radioactivity) (Table 76).

2. Study TEN-01-103: Drug-drug interaction study with midazolam (CYP3A4 substrate) and cefadroxil (PepT1 substrate)

As inhibition of NHE3 can lead to indirect inhibition of proton-coupled intestinal transporters such as peptide transporter 1 (PepT1), this study was to evaluate the NHE3 inhibition effect of tenapanor on cefadroxil (a PepT1 substrate). Since in vitro study results showed that tenapanor inhibited CYP3A4/5 with an IC_{50} of 0.4 µmol/L (458 ng/mL), the potential of tenapanor to interact with midazolam (CYP3A4/5 substrate) was also evaluated in this study.

Healthy subjects received single doses of cefadroxil 500 mg and midazolam syrup 7.5 mg alone, and received each drug again following tenapanor 50 mg BID for 12 and 13 days, respectively.

Point estimates and their 90% CI of AUC for cefadroxil and midazolam fell within the 80-125% criteria (Table 77) suggesting that tenapanor 50 mg BID does not significantly impact the PK of cefadroxil or midazolam. The drug interaction results indicated that tenapanor is not an inhibitor or inducer of CYP3A4/5 and does not inhibit PepT1 at the proposed clinical dose.

Table 77. Statistical Comparison of Cefadroxil and Midazolam Pharmacokinetics, with and without Concomitant Tenapanor

Comparison	N	Analyte	AUC_{last} Ratio% (90% CI)
Cefadroxil+Tenapanor vs. Cefadroxil alone	28	Cefadroxil	90.76 (86.65-95.07)
Midazolam+Tenapanor vs. Midazolam alone	28	Midazolam	116.04 (108.21-124.42)
		1-OH-midazolam	107.05 (100.73-113.76)
		4-OH-midazolam	111.98 (105.79-118.5)

Source: Study Report TEN-01-103, Table 11-6 and Table 11-7

Following repeated administration of tenapanor 50 mg BID for 12 or 13 days concomitantly with cefadroxil and midazolam, pharmacokinetic parameters of M1 are summarized in Table 78.

Table 78. Pharmacokinetic Parameters of M1 following repeated administration of tenapanor 50 mg BID with single oral cefadroxil 500 mg or midazolam 7.5 mg

Parameter (units)	Statistic	Cefadroxil + Tenapanor	Midazolam + Tenapanor
$AUC_{(0\text{-}12),ss}$, n=28	Mean (%CV)	152 (29.6)	168 (26.6)
(h*ng/mL)	Geometric mean	146	162
$AUC_{(0\text{-}last),ss}$, n=28	Mean (%CV)	152 (29.6)	168 (26.6)
(h*ng/mL)	Geometric mean	146	162
$C_{max,ss}$, n=28	Mean (%CV)	14.9 (29.4)	16.3 (27.8)
(ng/mL)	Geometric mean	14.3	15.7
$t_{max,ss}$, n=28	Median (range)	4.00 (0.00-12.02)	6.03 (2.00-12.22)
(h)			

Source: Study Report TEN-01-103, Table 11-5

3. Study TEN-01-104: Drug-drug interaction study with itraconazole (CYP3A inhibitor

In vitro study results showed that tenapanor is metabolized via CYP3A to M1. This study was designed to determine the effect of CYP3A4/5 inhibition by itraconazole on the PK of tenapanor and its primary metabolite, M1.

Fourteen healthy subjects received the following treatments:
- Days 1 and 6: single oral dose of 50 mg tenapanor
- Day 3: oral doses of 200 mg itraconazole BID
- Days 4 to 7: oral doses of 200 mg itraconazole QD

Arithmetic mean (standard deviation) trough concentrations on Days 6 through 8 ranged from 146 (52.1) ng/mL to 206 (55.1) ng/mL for itraconazole and from 407 (125) ng/mL to 528 (138) ng/mL for hydroxy itraconazole. These trough concentrations for itraconazole and hydroxy itraconazole were well above the reported half maximal inhibitory concentration values (approximately 29 nM [20.5 ng/mL] and 37 nM [26.7 ng/mL], respectively), indicating sufficient inhibition of CYP3A.

The PK parameters of tenapanor and M1 following a single oral dose of 50 mg tenapanor administered alone or with 200 mg itraconazole are summarized in Table 79 and Table 80.

Tenapanor exhibited minimal systemic bioavailability following a single oral dose of 50 mg tenapanor administered alone or with itraconazole. Following tenapanor administration alone, 3 of 14 subjects had a single quantifiable tenapanor concentration (~0.5 ng/mL) whereas more subjects (7 of 14) had higher quantifiable tenapanor concentrations (~1.0 ng/mL) following coadministration of tenapanor with itraconazole. Systemic exposure to M1 in terms of C_{max} and AUC_{last} decreased by approximately 33.9% to 46.6% following coadministration of tenapanor with itraconazole versus alone; Geometric mean ratios (90% CIs) for C_{max} and AUC_{last} were 53.4% (44.2, 64.5) and 66.1% (54.0, 80.8), respectively. These results are consistent with CYP3A inhibition by itraconazole as CYP3A4 and CYP3A5 are primarily responsible for the formation of M1.

Table 79. PK parameters of Tenapanor Following Single Dose of Tenapanor 50 mg with or without Itraconazole

Analyte: Tenapanor

	Treatment	
Parameter	Tenapanor (N=14[f])	Tenapanor plus Itraconazole (N=14[f])
AUC_{0-t} (h*ng/mL)	NC (NC)[c]	0.993 (27.3)[d]
$AUC_{0-\infty}$ (h*ng/mL)	NC (NC)[c]	NC (NC)[c]
C_{max} (ng/mL)	0.586 (12.1)[d]	0.843 (33.2)[e]
t_{max} (h)[a]	0.50 (0.50, 0.50)[d]	1.00 (0.50, 2.00)[e]
t_{last} (h)[a]	0.50 (0.50, 0.50)[d]	1.50 (1.00, 2.00)[e]
$t_{1/2}$ (h)[b]	NC (NC)[c]	NC (NC)[c]
CL/F (L/h)	NC (NC)[c]	NC (NC)[c]
V_z/F (L)	NC (NC)[c]	NC (NC)[c]

Note: Geometric mean (CV%) data are presented; [a]Median (min , max); [b]Arithmetic mean (SD); [c]N = 0; [d]N = 3; [e]N = 7; [f]Total number of subjects who completed the study, including subjects with PK data that were not evaluable.
Source: Study Report TEN-01-104, Table 5.

Table 80. PK parameters of M1 Following Single Dose of Tenapanor 50 mg with or without Itraconazole

Analyte: AZ13792925

Parameter	Treatment	
	Tenapanor (N=14[g])	Tenapanor plus Itraconazole (N=14[g])
AUC_{0-t} (h*ng/mL)	312 (35.5)	195 (45.8)[f]
$AUC_{0-\infty}$ (h*ng/mL)	378 (37.6)	198 (14.0)[d]
C_{max} (ng/mL)	13.2 (39.8)	6.69 (44.3)[f]
t_{max} (h)[a]	4.00 (3.00, 8.00)	4.00 (3.00, 16.00)[f]
t_{last} (h)[a]	47.33 (47.33, 47.50)	48.00 (48.00, 48.00)[f]
$t_{1/2}$ (h)[b]	18.7 (3.74)	30.3 (12.4)[f]
MR_{AUC}	NC (NC)[c]	553 (49.4)[d]
MR_{Cmax}	60.5 (32.8)[d]	20.1 (63.3)[e]

[e]N = 5; [f]N = 10; [g]Total number of subjects who completed the study, including subjects with PK data that were not evaluable.
Source: Study Report TEN-01-104, Table 6.

4. Study D5611C00005: The pharmacokinetics and pharmacodynamics of tenapanor capsules in Japanese and Caucasian Healthy Subjects

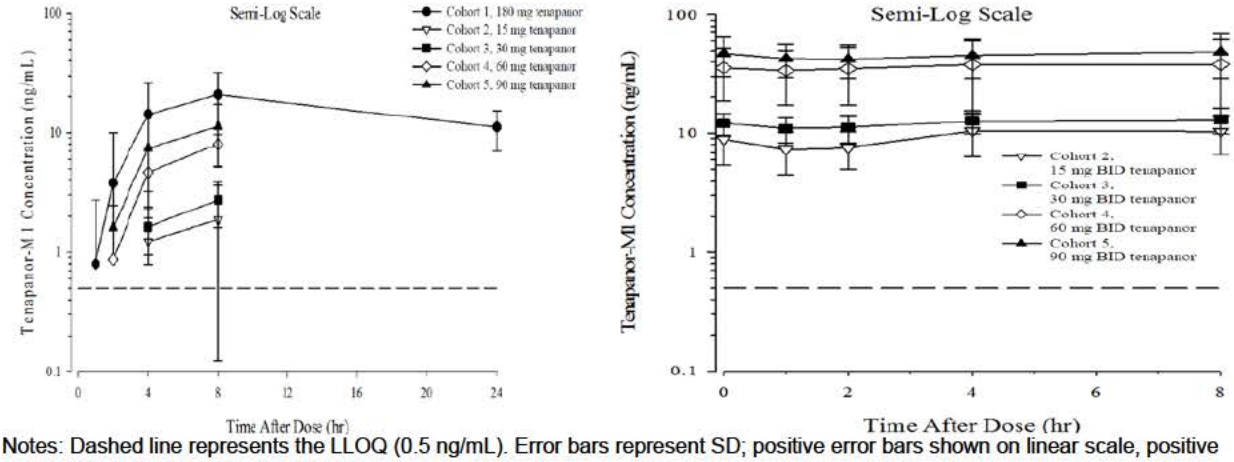
This is a double-blind, randomized, placebo-controlled, multiple-dose study in which 83 healthy adult Japanese and Caucasian subjects received either a single dose of tenapanor capsules 180 mg, or twice daily ascending doses of tenapanor capsules 15 to 90 mg (14 to 88 mg) in order to assess the pharmacokinetics and pharmacodynamics of tenapanor in Caucasian and Japanese healthy subjects.

- Cohort 1- Japanese: Tenapanor capsules 60 mg*3 (180 mg), single dose, n=6
- Cohort 2- Japanese: Tenapanor capsules 15 mg (14 mg), BID for 7 days, n=12
- Cohort 3- Japanese: Tenapanor capsules 30 mg (28 mg), BID for 7 days, n=12
- Cohort 4- Japanese: Tenapanor capsules 60 mg (60 mg), BID for 7 days, n=12
- Cohort 5- Japanese: Tenapanor capsules 90 mg (88 mg), BID for 7 days, n=12
- Cohort 6- Caucasian: Tenapanor capsules 90 mg (88 mg), BID for 7 days, n=12

Pharmacokinetics

All but three of 270 post-dose pharmacokinetic samples (99% of samples) were below the limit of quantitation for parent tenapanor (< 0.5 ng/mL). Mean plasma concentration-time profiles for M1 on Days 1 and 7 in Japanese subjects are presented in Figure 9. Maximum M1 concentrations were generally reached by 4–8 hours after administration. Over the dose range of 15-90 mg (14-88 mg), C_{max} and AUC_{0-8h} of M1 generally increased proportionally to tenapanor dose. When administered as multiple doses, accumulation in C_{max} of M1 is approximately 5-fold. M1 exposure appeared generally comparable for Japanese and Caucasian subjects after administration of tenapanor capsules at 90 mg BID (Table 81).

Figure 9. Mean (± SD) Plasma Concentration Versus Time Profiles for M1 after Single Dose (Left) and Repeated Doses for 7 Days (Right) in Japanese Subjects



Notes: Dashed line represents the LLOQ (0.5 ng/mL). Error bars represent SD; positive error bars shown on linear scale, positive and negative shown on semi-log scale.
Source: Repot D5611C00005- (b) (4) Study Number 8329642 (metabolite report), Figure 1 and Figure 2.

Table 81. Arithmetic Mean (SD) Pharmacokinetic Parameters for M1 in Japanese and Caucasian Subjects

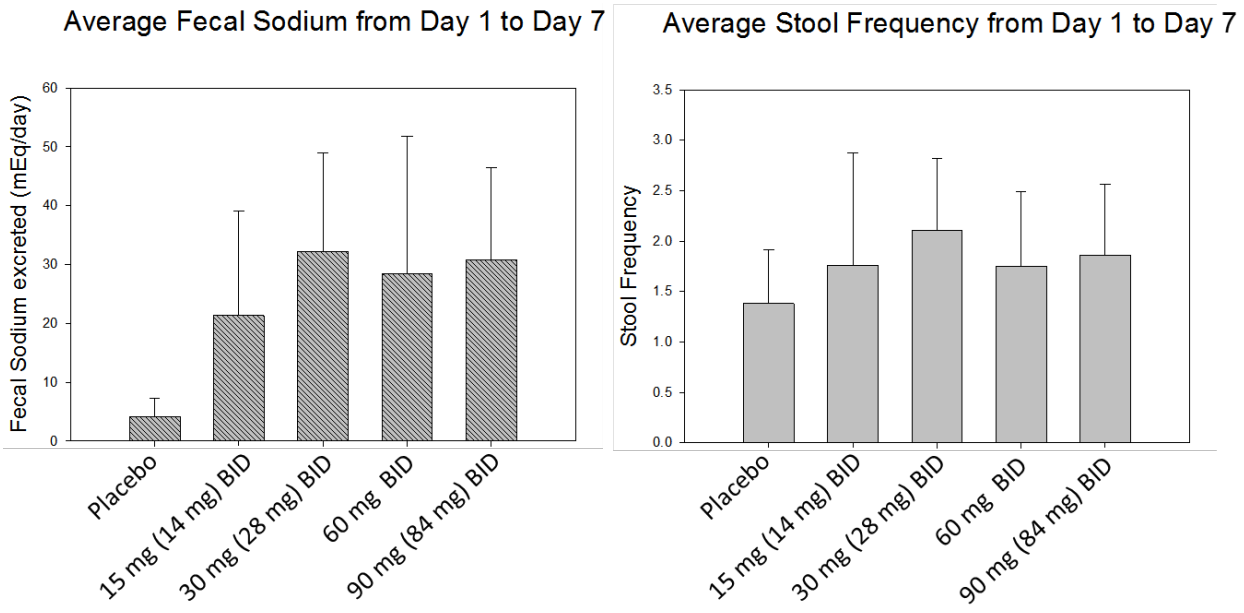
Parameter	Dose of Tenapanor										
	Cohort 1 180 mg	Cohort 2 15 mg BID		Cohort 3 30 mg BID		Cohort 4 60 mg BID		Cohort 5 90 mg BID		Cohort 6[c] 90 mg BID	
	Day 1 (N=6)	Day 1 (N=12)	Day 7 (N=12)	Day 1 (N=12)	Day 7 (N=12)	Day 1 (N=12)	Day 7 (N=12)	Day 1 (N=12)	Day 7 (N=12)	Day 1 (N=12)	Day 7 (N=12)
C_{max} (ng/mL)	21.4 (10.2)	1.88 (1.77)	11.0 (4.04)	2.73 (1.12)	13.8 (2.88)	7.99 (2.82)	40.6 (22.9)	11.2 (6.00)	52.7 (20.8)	9.48 (4.26)	52.1 (16.9)
T_{max}[a] (hr)	8.00 (8.00, 24.0)	8.00 (4.00, 8.00)	4.03 (0, 8.03)	8.00 (8.00, 8.02)	4.11 (0, 8.03)	8.00 (8.00, 8.05)	4.00 (0, 8.00)	8.00 (7.98, 8.00)	4.00 (0, 8.03)	8.00 (8.00, 8.15)	8.00 (0, 8.05)
$AUC_{0\text{-}8}$ (hr·ng/mL)	90.6 (67.6)	9.75 (11.6)[b]	75.0 (26.1)	10.7 (4.06)	98.0 (21.2)	31.4 (14.1)	293 (167)	47.1 (34.0)	364 (127)	36.8 (17.8)	340 (110)

Source: Repot D5611C00005- (b) (4) Study Number 8329642 (metabolite report), Table 2.

Pharmacodynamics

Average daily fecal sodium and phosphorous excretion from Day 1 to Day 7 was greater after administration of all doses of tenapanor compared to placebo, but apparent dose-response relationship was not observed at doses greater than 30 mg BID (Figure 10, **left**). Of note, subjects were given a standardized diet with an approximate daily sodium content of 4500 mg. Daily stool frequency and daily stool weight slightly increased after repeated dosing of 15-90 mg BID tenapanor compared to placebo (Figure 10, **right**).

Figure 10. Fecal Sodium Excretion (left) and Stool Frequency (right) by Dose after Repeated Dosing for 7 Days in Japanese Subjects



Source: Reviewer's plot based on Study Report D5611C00005, Table 6 and Table 7.

Potential QT prolongation

The Applicant extensively monitored ECG in this study and evaluated potential QT prolongation (Study D5611C0005, Cardiac Safety Report). Per IRT-QT review dated Jan 25, 2019, no significant QTc prolongation effect of tenapanor was detected in the QTc assessment up to tenapanor capsule 90 mg BID which resulted in the maximum exposure (C_{max}) to M1 of 48.7 ng/mL. See IRT-QT review in DARRTS dated Jan 25, 2019 for more information. The M1 concentration was approximately 3-fold greater than that reported in healthy subjects taking tenapanor 50 mg BID in the to-be-marketed formulation and midazolam, i.e., 16.3 ng/mL, in Study TEN-01-103.

5. Study D5611C00003 (Part A): Effect of food on pharmacodynamics of tenapanor

This study evaluated the effect of different dosing timing in relations to meals on pharmacodynamics of tenapanor. In this three-way crossover, open label study, a total of 19 healthy male and female adult subjects received the following three treatments for 4 days each, separated by a drug-free washout period of two days:

- Treatment A: Tenapanor Tablets 15 mg (14 mg) twice daily, immediately prior to meals, i.e., 5 to 10 minutes before start of food intake
- Treatment B: Tenapanor Tablets 15 mg (14 mg) twice daily, after meals, i.e., 30 minutes after start of food intake
- Treatment C: Tenapanor Tablets 15 mg (14 mg) twice daily, in a fasting state, i.e., 1 hr before or 3 hrs before start of food intake

Fecal samples were collected at baseline, and on Study Days 1-4 for pharmacodynamic assessments of fecal sodium and phosphorous excretion. Subjects were served with a diet standardized for sodium content while resident in the study center.

The mean amounts of sodium and phosphorous (mEq) excreted in the feces per day during each treatment period are presented in Table 82. The mean 24-hour fecal sodium was approximately 8 mEq/day and 11 mEq/day higher when tenapanor was given before a meal (Treatment A) in comparison to when tenapanor was given after a meal (Treatment B) and in fasting conditions (Treatment C), respectively. The mean 24-hour fecal phosphorous excreted showed similar trend of effect as the fecal sodium.

All pharmacokinetic samples were below the limit of quantitation for parent tenapanor using a validated LC-MS/MS method with a lower limit of quantification of 0.5 ng/mL.

Table 82. Statistical Analysis of Fecal Sodium and Phosphorous (mEq & grams/day) Excreted Following Administration of Tenapanor Tablets Prior to Meals, Fed and Fasting

						Pairwise Comparisons		
Parameter (units)	Trt	N	n	LS mean	95% CI	Pair	Diff	90% CI	p-value
Sodium (mEq/day)	A	18	8	25.9388	(19.4989, 32.3788)	A vs B	8.7767	(3.7295, 13.8240)	0.0060
	B	18	18	17.1621	(10.7221, 23.6021)	B vs C	3.0439	(-2.0034, 8.0911)	0.3147
	C	18	18	14.1182	(7.6783, 20.5582)	A vs C	11.8206	(6.7733, 16.8679)	0.0004
Sodium (g/day)	A	18	18	0.5961	(0.4481, 0.7441)	A vs B	0.2017	(0.0857, 0.3177)	.0060
	B	18	18	0.3944	(0.2464, 0.5424)	B vs C	0.0699	(-0.0460, 0.1859)	0.3147
	C	18	18	0.3244	(0.1764, 0.4724)	A vs C	0.2716	(0.1557, 0.3876)	0.0004

Diff Difference; Trt Treatment

Treatment A: AZD1722 administered 5 to 10 minutes before start of intake of breakfast and dinner

Treatment B: AZD1722 administered 30 minutes after start of intake of breakfast and dinner

Treatment C: AZD1722 administered 1 hour before breakfast and 3 hours after start of intake of dinner and 1 hour before next meal consumption

Source: Study Report D5611C00003, Part A, Table 7

6. Study D5612C00001 (phase 2b): Tenapanor in patients with irritable bowel syndrome with constipation

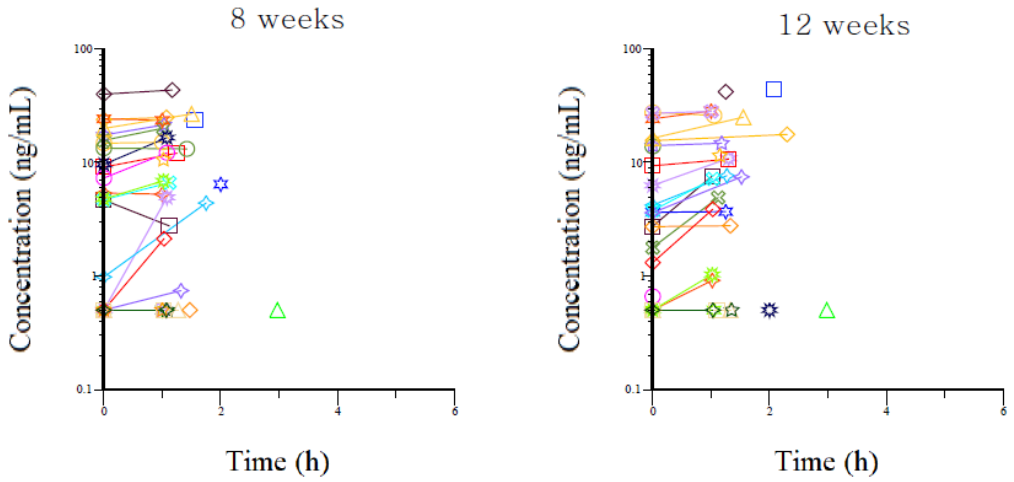
In this Phase 2, randomized, double-blind, placebo-controlled study, 356 IBS-C patients were randomized 1:1:1:1 to receive placebo (n=90), tenapanor tablets 5 mg (n=88), tenapanor tablets 20 mg (n=89) or tenapanor tablets 50 mg (n=89) BID for 12 weeks. A total of 304 subjects completed the study according to the protocol: placebo (n=80), tenapanor 5 mg (n=69), tenapanor 20 mg (n=77), and tenapanor 50 mg (n=78).

Blood samples for tenapanor and M1 pharmacokinetic analysis were collected from a subset of approximately 30 subjects per treatment group at the 8- and 12-week site visits. PK samples were collected 1-4 hours (5 hours in one subject) after the morning dose of tenapanor. A pre-dose sample was also collected when possible.

Tenapanor showed minimal systemic availability. Eight of the 291 (2.7%) subjects had plasma tenapanor concentrations of 0.547-1.03 ng/mL, and the concentrations were below the lower limit of quantification (< 0.5 ng/mL) in other subjects.

Plasma concentration-time profiles for M1 after 8 and 12 weeks of treatment are presented in Figure 11. Observed plasma M1 concentrations are summarized in Table 83. Approximately 32% [94 of 296] of analyzed samples had plasma M1 concentrations below the limit of quantitation (< 0.5 ng/mL).

Figure 11. Plasma M1 Concentration-Time Profiles of Individual Patients with IBS-C Administered 50 mg BID Doses of Tenapanor for 8 and 12 Weeks



Source: D5612C00001-RDX5791-PK-20 (metabolite report), Figure 3

Table 83. Plasma M1 Concentrations Observed after Multiple Dose Administration of Tenapanor 5, 20 and 50 mg BID

Dose	Study Day	N	Concentration range (ng/mL)	Mean maximum concentration (SD) (ng/mL)
5 mg BID	57	29	BQL – 9.11	2.16 (1.85)
	85	25	BQL – 7.94	2.83 (2.08)
20 mg BID	57	27	BQL – 22.5	4.47 (5.73)
	85	25	BQL – 18.3	4.87 (5.18)
50 mg BID	57	31	BQL – 43.6	10.1 (10.7)
	85	30	BQL – 43.9	10.9 (12.5)

BQL: below quantitative limit (< 0.5 ng/mL)
Source: D5612C00001- RDX5791-PK-20 (metabolite report), Table 5

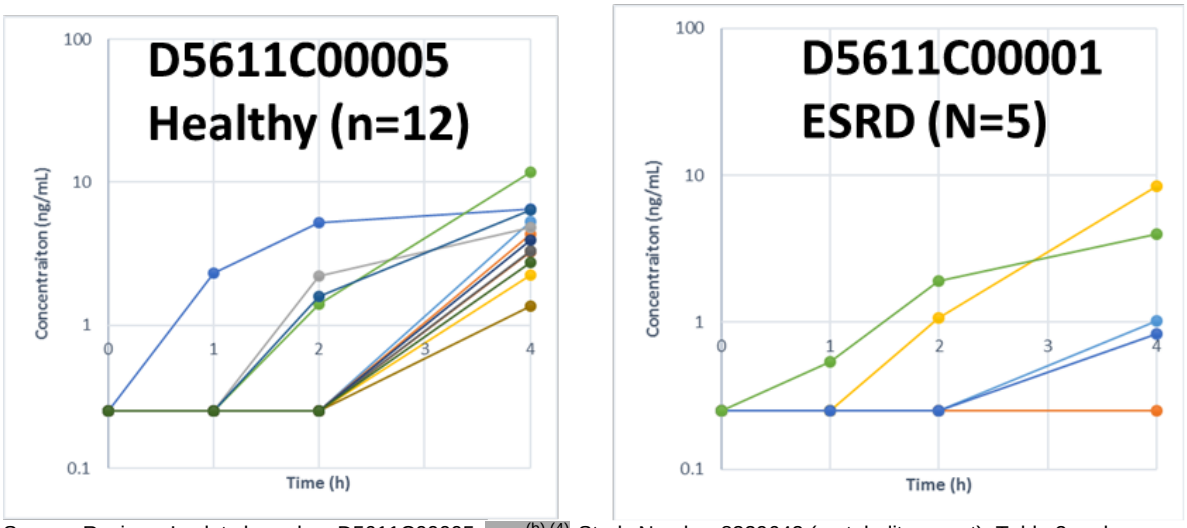
7. Study D5611C00001: Tenapanor in patients with end-stage renal disease

In this Phase 2a, randomized, double-blind, placebo-controlled study, 88 end-stage renal disease patients (eGFR < 15 mL/min/1.73m^2) on hemodialysis were randomized to receive

(b) (4)

Based on cross-study comparison (D5611C00001 in ESRD patients vs. D5611C00005 in healthy subjects), following single dose of tenapanor (b) (4)), the mean maximum plasma M1 concentrations were lower in ESRD patients, i.e., 2.55 ng/mL (n=5) vs. 7.99 ng/mL (n=12) (**Figure 12.**).

Figure 12. Plasma M1 Concentration-Time Profiles of Individual Healthy Subjects (Left) and ESRD Patients (right) After Single Dose of 60 mg (56 mg) Tenapanor Capsule



Source: Reviewer's plots based on D5611C00005- (b) (4) Study Number 8329642 (metabolite report), Table 2 and D5611C00001- RDX5791-PK-19 (metabolite report), Tables 9

16.3.2. In Vitro Drug-Drug Interaction Studies

Substrate of transporters

Tenapanor was not a substrate of P-gp, BCRP, OATP1B1, and OATP1B3 (Table 84). Since tenapanor was not excreted by the kidney, the potential of M1 as a substrate of renal transporters, was not tested.

M1 was not a substrate of BCRP, OAT1, OAT3, OCT2, MATE1, and MATE2-K (Table 85). It was a weak substrate of P-gp (Table 85). Since the intrinsic clearance of M1 was very low in human

liver microsomes, the potential of M1 as a substrate of the hepatic uptake transporters, OATP1B1 and OATP1B3, was not tested.

Table 84. In Vitro Studies to Determine the Transporters of Tenapanor

Characterization of cell permeability: **tenapanor permeability is low.**	RDX5791-PK-09 (MDCK): P_{app} ≤0.02 ×10^{-6} cm/sec in A-B and B-A directions
Characterization as a human P-gp substrate: **tenapanor is not a substrate of human P-gp.**	ADME-ADX-170825-P-gp Substrate (MDCKII-MDR1): P_{app} ≤ 0.728 ×10^{-6} cm/sec in both directions ± P-gp inhibitor
Characterization as a P-gp inhibitor: **tenapanor is not an inhibitor of P-gp.**	Pgp inhib_13Dec12_AZ13667691 (MDCKII-MDR1): B-A P_{app} values for [^{3}H]-digoxin ≥13.2 ×10^{-6} cm/sec with 0.3-100 µM tenapanor
Characterization as a BCRP substrate: **tenapanor is not a substrate of BCRP**	ADME-ADX-170825-BCRP Substrate (Caco-2) P_{app} ≤ 1.10 ×10^{-6} cm/sec in both directions ± BCRP inhibitor with similar efflux ratios
Characterization as a BCRP inhibitor: **tenapanor is not an inhibitor of BCRP.**	13ASTRUKP1 (BCRP-MDCK): B-A P_{app} of cladribine was 13.5-19.9 ×10^{-6} cm/sec with 1-300 µM tenapanor
Characterization as an OATP1B1/3 substrate: **tenapanor is not a substrate of OATP1B1 and OATP1B3**	ADME-ADX-170825-SLC Substrate (HEK293): estradiol-17β-glucuronide uptake by OATP1B1 and OATP1B3 was reduced by 13.9% and 16.2% with inhibitor, respectively and uptake ratio was <2
Characterization as an OATP1B1/3 transporter inhibitor: **tenapanor is an inhibitor of OATP1B1 and OATP1B3 mediated transport**	ADME-ADX-170825-SLC Inhibition (HEK293): OATP1B1- and OATP1B3-mediated transport of estradiol-17β-glucuronide inhibited by tenapanor with IC_{50} of 0.658 µM and 1.43 µM, respectively

Source: 2.7.2. Summary of Clinical Pharmacology, Section 2.7.2.2.1.1.4

Table 85. In Vitro Studies to Determine the Transporters of M1 (AZ13792925)

Study/ Protocol No.	Transporter (Test System)	Concentration of M1 Tested	M1 efflux ratio	AZ13792925 + inhibitor efflux ratio
ADME-ADX-170824-P-gp Substrate	P-gp (MDCKII-MDR1 cells)	5 µM	2.39 ± 0.105	0.615 ± 0.0375 (verapamil)
ADME-ADX-170824-BCRP substrate	BCRP (Caco-2 cells)	5 µM	0.718 ± 0.0444	0.703 ± 0.0457 (novobiocin)
ADME-ADX-170824-SLC Substrate	OAT1, OAT3, OCT2, MATE1, MATE2K (HEK293 cells)	5 µM	<1.08	<1.12

Source: 2.7.2. Summary of Clinical Pharmacology, Table A-10

Inhibition of transporters

Tenapanor or M1 did not inhibit human P-gp, BCRP, OATP1B1 and OATP1B3 indicating that the potential for an in vivo DDI was low (Table 86, Table 87). M1 did not inhibit OAT1, OAT3, OCT2, MATE1, and MATE2-K (Table 87).

Table 86. In Vitro Studies to Characterize the Inhibition of Transporters of Tenapanor

Study/ Protocol No.	Transporter (Test System)	Concentration Range of Tenapanor Tested	Probe Substrate	Reference Inhibitor	Inhibition by Reference	Inhibition by Tenapanor
Pgp inhib_13Dec12_AZ13667691	P-gp (MDCKII-MDR1 cells)	0.3, 1, 3, 10, 30 and 100 µM	[^{3}H]-Digoxin (5 µM)	Verapamil (1, 3, 10, 30, 100 and 300 µM)	6.4 (IC_{50}, µM)	No inhibition
13ASTRUKP1	BCRP (BCRP-MDCK cells)	1, 3, 10, 30, 100 and 300 µM	Cladribine (10 µM)	Ko143 (10 µM)	100%	≤ 20.3%
ADME-ADX-170825-SLC Inhibition	OAT1B1 & OAT1B3 (HEK293 cells)	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM	Estradiol-17β-glucuronide (for OATP1B1, 5 µM; for OATP1B3, 10 µM)	Rifampicin SV (0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µM)	OAT1B1- 0.142 (IC_{50}, µM) OAT1B3- 0.192 (IC_{50}, µM)	OAT1B1- 0.658 (IC_{50}, µM) OAT1B3- 1.43 (IC_{50}, µM)

Source: 2.7.2. Summary of Clinical Pharmacology, Table A-9

Table 87. In Vitro Studies to Characterize the Inhibition of Transporters of M1 (AZ13792925)

Study/ Protocol No.	Transporter (Test System)	Concentration Range of AZ13792925 Tested	Probe Substrate	Reference Inhibitor (s)	Inhibition by Reference(s) (IC_{50}, µM/% inhibited)	Inhibition by AZ13792925 (IC_{50}, µM)
ADME-AZS-Wave3-150412	P-gp (MDCKII-MDR1 cells)	0.3, 1, 3, 10, 30 and 100 µM	Digoxin (5 µM)	Verapamil	3.56	80.7
ADME-AZS-Wave3-150413	BCRP (Caco-2 cells)	0.3, 1, 3, 10, 30 and 100 µM	Rosuvastatin (1 µM)	Novobiocin	1.21	43.7
ADME-AZS-Wave3-150415	OATP1B1 (HEK293-OATP1B1 Cells)	0.3, 1, 3, 10, 30 and 100 µM	Estradiol 17β glucuronide	Rifamycin SV Erythromycin	0.0995 30.6	23.6
15ASTRUKP1S4	OAT1, OAT3, OCT2, OATP1B3, MATE1 and MATE2K (transfected HEK293 cells)	0.3, 1, 3, 10, 30 and 100 µM	OAT1- PAH OAT3- Furosemide OCT2- Metformin. OATP1B3- Atorvastatin MATE1- Metformin MATE2K- Metformin	OAT1- Probenecid OAT3- Probenecid OCT2- Imipramine OATP1B3- Rifamycin SV MATE1- Cimetidine MATE2K- Pyrimethamine	OAT1- 89.9 % OAT3- 97.9% OCT2- ≥98.3% OATP1B3- 96.1% MATE1- 98.4% MATE2K- 92.0%	OAT1->100 OAT3- none OCT2- N.D. OATP1B3- 46.7 MATE1- 16.9 MATE2K- 21.9

Source: 2.7.2. Summary of Clinical Pharmacology, Table A-11

Metabolism by CYP enzymes

Tenapanor was a substrate of CYP3A4 and CYP3A5. However, none of the CYP enzymes was found to be responsible for ≥ 25% of its overall elimination. The metabolic stability of M1 was tested at 1 µM in human liver microsomes incubated with NADPH for up to 30 minutes. M1 was stable, with $t_{1/2}$ and CL_{int} values of 91.5 minutes and 16.3 µL/min/mg protein, respectively, indicating that M1 was a weak substrate of Phase I metabolism enzymes in the liver. In clinical studies, Phase I or II metabolites of M1 were not detected in plasma, urine, or feces from the human [^{14C}] tenapanor ADME study (D5611C00007) and the plasma concentration-time profile of M1 at steady state was flat, suggesting that the in vivo clearance of M1 is very low (Table 88).

Table 88. In Vitro Studies to Identify the CYP Isozyme Responsible for the Metabolism of Tenapanor

Study/ Protocol No.	Test System	Concentration of Tenapanor Tested	Intrinsic Clearance (µL/min/pmol CYP) (for CYP >1 µL/min/pmol CYP)	% of total metabolism (For CYP > 1% of total)	Formation velocity of M1 (pmol/min/nmol CYP) after a 5 minute incubation (For CYP >10 pmol/min/nmol CYP)
ADME-AZS-Wave3 (CYP isoform ID)-150527	Bactosomes expressing individual human CYP isoforms + NADPH	2 µM	CYP3A4- 9.11±0.821 CYP3A5- 8.45±0.425 CYP2C8- 3.14±0.155 CYP2D6- 1.64±0.303	CYP3A4- 56.8 CYP3A5- 39.8 CYP2C8- 2.19	CYP3A4- 339±27.2 CYP3A5- 245±14.7 CYP2C8- 19.1±2.37 CYP2D6- 12.5±6.42

Source: 2.7.2. Summary of Clinical Pharmacology, Table A-2

Inhibition of CYP enzymes

Neither tenapanor nor M1 inhibited CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 (Table 89).

Table 89. CYP450 Reversible inhibition (IC_{50}) by Tenapanor and M1 (AZ13792025)

CYP Isoform	Tenapanor		AZ13792925		In vivo DDI study needed?
	IC_{50} (µM)	R_1 (or $R_{1,gut}$)	IC_{50} (µM)	R_1 (or $R_{1,gut}$)	
CYP1A2	NI	NC	NI	NC	No
CYP2A6	NT	NT	NI	NC	No
CYP2B6	15.4	1.000001	>30	<1.0001	No
CYP2C8	10.4	1.000002	NI	NC	No
CYP2C9	14.2	1.000002	NI	NC	No
CYP2C19	>30	<1.0000008	>30	<1.0001	No
CYP2D6	3.26	1.000007	1.95	1.002	No
CYP2E1	NT	NT	NI	NC	No
CYP3A4 (midazolam)	0.402	1.000056 (1739)	0.0813	1.047 (8595)	Yes
CYP3A4 (nifedipine)	0.68	1.000033 (1028)	0.11	1.035 (6353)	

NI=No inhibition; NC=Not calculated; NT: Not tested

Source: 4.2.2.7. Study report of RDX5791-pk-16, Table 4

Induction of CYP enzymes

Neither tenapanor nor M1 induced CYP1A2 and CYP2B6 (Table 90).

Table 90. CYP450 Induction by Tenapanor and M1 (AZ13792025)

CYP Isoform	Tenapanor				AZ13792925				In vivo DDI study needed?
	E_{max}	EC_{50} (µM)	F2 (µM)	R_3	E_{max}	EC_{50} (µM)	F2 (µM)	R_3	
CYP1A2	NI	NI	NI	NC	NI	NI	NI	NC	No
CYP2B6	NI	NI	NI	NC	>4.27 (NI, 2/3 donors)	NC	0.766	NC	No
CYP3A4	5.53 (NI, 3/4 studies)	NC	NC	NC	4.29, 15.9 (NI, 1/3 donors)	0.988, 1.24	1.00, 0.565	0.768	Yes

NI=No induction; NC=Not calculated

Source: 4.2.2.7. Study report of RDX5791-pk-16, Table 6

16.3.3. Summary of Bioanalytical Method Validation and Performance

Tenapanor concentrations in plasma and urine

Plasma and urine concentrations of tenapanor were measured using validated bioanalytical methods with LC-MS/MS. The performance and validation parameters of these methods are not included in this review because most concentrations of tenapanor were below LLOQ (<0.5 ng/mL).

Major metabolite M1 (AZ13792925) concentrations in plasma

The performance and validation parameters of the bioanalytical method "AZ17HPP" for measuring plasma M1 concentrations are summarized in Table 91.

Table 91. Summary of the performance and validation parameters of the bioanalytical method to measure M1 in plasma (Method ID: AZ17HPP)

Bioanalytical method review summary	**Method validation was adequate to support the study TEN-01-103 and TEN-01-104**		
Method description	Drug extraction from K2EDTA human plasma by protein precipitation and quantitation with LC-MS/MS method		
Materials used for calibration curve & concentration	human plasma, K2EDTA		
Validated assay range	0.500 to 375 ng/mL		
Material used for QCs & concentration	human plasma, K2EDTA		
Regression model & weighting	Linear regression, $1/x^2$		
Validation parameters	**Method validation summary**		**Acceptability**
Calibration curve performance during accuracy & precision	No of standard calibrators from LLOQ to ULOQ	8	Yes
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.4 to 2.7%	Yes

Bioanalytical method review summary	Method validation was adequate to support the study TEN-01-103 and TEN-01-104		
	Cumulative precision (%CV) from LLOQ to ULOQ	≤7.8%	Yes
QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 4 QCs	-8.0 to -2.0%	Yes
	Inter-batch %CV	≤7.9%	Yes
	Percent total error (TE)	0%	Yes
Selectivity & matrix effect/ Interference & specificity	Six individual blank matrix samples tested. No interference peaks (>20.0% of the LLOQ calibration standard response; >5.0% of the internal standard peak response) The blank samples spiked with tenapanor did not contain any peaks at the retention time of M1 or internal standard.		Yes
Hemolysis effect	Total of 6 plasma lots tested. %bias ranged from -11.3 to 7.7%.		Yes
Lipemic effect	Total of 6 plasma lots tested. %bias ranged from 0.7 to 5.3%.		Yes
Dilution linearity & hook effect	Linear within 25-fold dilutions. Tested at 7500 ng/mL. No hook effect		Yes
Bench-top/process stability	Stable at room temperature for 24 hours in plasma Stable at 2 to 8℃ for 110 hours in processed-sample		Yes
Freeze-Thaw stability	Up to 5 cycles at -10 to -30℃ and -60 to -80℃		Yes
Long-term storage	Stable for 48 days at -10 to -30°C or at -60 to -80°C		Yes
Carry over	No evidence of carryover		Yes
	Method performance in study TEN-10-103		
Assay passing rate	8 out of 9 runs (including incurred sample reanalysis (ISR)) met the method acceptance criteria.		Yes
Standard curve performance	%bias: -1.8 to 3.0; %CV ≤7.0%		Yes
QC performance	At 1.5, 20.0, and 375 ng/mL, %bias: -2.7 to 0.5; %CV ≤5.7%		Yes
Method reproducibility	40 samples reanalyzed and 98% of the repeat results and original results were within 20.0% of the mean of the two values		Yes
Study sample analysis/ stability	All samples were analyzed within 48 days of collection following storage at -60 to -80°C within established stability		Yes
	Method performance in study TEN-10-104		
Assay passing rate	8 out of 8 runs (including incurred sample reanalysis (ISR)) met the method acceptance criteria.		Yes
Standard curve performance	%bias: -5.8 to 4.7; %CV ≤9.0%		Yes
QC performance	At 1.5, 20.0, and 375 ng/mL, %bias: -1.1 to 8.0; %CV ≤5.7%		Yes
Method reproducibility	46 samples reanalyzed and 98% of the repeat results and original results were within 20.0% of the mean of the two values		Yes
Study sample analysis/ stability	All samples were analyzed within 48 days of collection following storage at -60 to -80°C within established stability		Yes

Source: Validation Report of method AZ17HPP (b) (4) Study Number 8316236); Bioanalytical Report of TEN-10-103; Bioanalytical Report of TEN-10-104

Midazolam, 1'-hydroxymidazolam, and Cefadroxil Plasma Concentrations (TEN-10-103)

Plasma samples obtained in Study TEN-10-103 were analyzed for midazolam, 1'-hydroxymidazolam, 4'-hydroxymidazolam and cefadroxil concentrations by (b) (4) using LC-MS/MS. The validated ranges of quantitation were 0.1 to 100 ng/mL for midazolam, 1'-dydroxymidazolam, and 4'-hydroxymidazolam. The validated range of quantitation was 30 to 30,000 ng/mL for cefadroxil. Method validations were adequate to support the study TEN-01-103 ((b) (4) Study Number: 8280288 for midazolam and its metabolites; 8298908 for cefadroxil). All samples were analyzed within 48 days of collection following storage at -60 to -80°C within established long-term stability.

Itraconazole, hydroxy-itraconazole Plasma Concentrations (TEN-10-104)

Plasma samples obtained in Study TEN-10-104 were analyzed for itraconazole and hydroxy-itraconazole concentrations by (b) (4) using LC-MS/MS. The validated ranges of quantitation were 2.0 to 1,000 ng/mL for midazolam, 1'-dydroxymidazolam, and 4'-hydroxymidazolam. Method validation was adequate to support the study TEN-01-104 ((b) (4) Study Number: 8390863). All samples were analyzed within 48 days of collection following storage at -60 to -80°C within established long-term stability.

Sodium in human feces

The amounts of sodium were measured in fecal samples in clinical studies including D5611C00005 (PK-PD and QT assessment) and D5611C00003 (food effect study). The quantitation range was from 0.25 to 25 µg/mL. Samples were processed employing a partial digestion of the entire sample in nitric acid (pre-digest) followed by a more complete and higher temperature digestion of a subsample of the pre-digest using nitric and hydrochloric acids. The digest was then centrifuged, and the sodium content of the supernatant liquid was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES).
The total sodium amounts (in grams) were calculated using the processed sample volume and the measured concentration. The total milliequivalents (mEq) per day were calculated from the total grams of each individual analyte per day.

16.4. References

Amin, MR, J Malakooti, R Sandoval, PK Dudeja, and K Ramaswamy, 2006, IFN-gamma and TNF-alpha regulate human NHE3 gene expression by modulating the Sp family transcription factors in human intestinal epithelial cell line C2BBe1, Am J Physiol Cell Physiol, 291(5):C887-896.

Barmeyer, C, M Harren, H Schmitz, U Heinzel-Pleines, J Mankertz, U Seidler, I Horak, B Wiedenmann, M Fromm, and JD Schulzke, 2004, Mechanisms of diarrhea in the interleukin-2-deficient mouse model of colonic inflammation, Am J Physiol Gastrointest Liver Physiol, 286(2):G244-252.

Black, CJ, NE Burr, EMM Quigley, P Moayyedi, LA Houghton, and AC Ford, 2018, Efficacy of Secretagogues in Patients With Irritable Bowel Syndrome With Constipation: Systematic Review and Network Meta-analysis, Gastroenterology, 155(6):1753-1763.

Chalasani, N, HL Bonkovsky, R Fontana, W Lee, A Stolz, J Talwalkar, KR Reddy, PB Watkins, V Navarro, H Barnhart, J Gu, J Serrano, and United States Drug Induced Liver Injury Network, 2015, Features and Outcomes of 899 Patients With Drug-Induced Liver Injury: The DILIN Prospective Study, Gastroenterology, 148(7):1340-1352 e1347.

Donowitz, M, C Ming Tse, and D Fuster, 2013, SLC9/NHE gene family, a plasma membrane and organellar family of Na(+)/H(+) exchangers, Mol Aspects Med, 34(2-3):236-251.

Enck, P, Q Aziz, G Barbara, AD Farmer, S Fukudo, EA Mayer, B Niesler, EM Quigley, M Rajilic-Stojanovic, M Schemann, J Schwille-Kiuntke, M Simren, S Zipfel, and RC Spiller, 2016, Irritable bowel syndrome, Nat Rev Dis Primers, 2:16014.

Engevik, MA, KA Engevik, MB Yacyshyn, J Wang, DJ Hassett, B Darien, BR Yacyshyn, and RT Worrell, 2015, Human Clostridium difficile infection: inhibition of NHE3 and microbiota profile, Am J Physiol Gastrointest Liver Physiol, 308(6):G497-509.

Girardi, AC and F Di Sole, 2012, Deciphering the mechanisms of the Na+/H+ exchanger-3 regulation in organ dysfunction, Am J Physiol Cell Physiol, 302(11):C1569-1587.

Gurney, MA, D Laubitz, FK Ghishan, and PR Kiela, 2017, Pathophysiology of Intestinal Na(+)/H(+) exchange, Cell Mol Gastroenterol Hepatol, 3(1):27-40.

Gwee, KA, CL Lu, and UC Ghoshal, 2009, Epidemiology of irritable bowel syndrome in Asia: something old, something new, something borrowed, J Gastroenterol Hepatol, 24(10):1601-1607.

Harrison, CA, D Laubitz, CL Ohland, MT Midura-Kiela, K Patil, DG Besselsen, DR Jamwal, C Jobin, FK Ghishan, and PR Kiela, 2018, Microbial dysbiosis associated with impaired intestinal Na(+)/H(+) exchange accelerates and exacerbates colitis in ex-germ free mice, Mucosal Immunol, 11(5):1329-1341.

Hayashi, H, K Szaszi, N Coady-Osberg, W Furuya, AP Bretscher, J Orlowski, and S Grinstein, 2004, Inhibition and redistribution of NHE3, the apical Na+/H+ exchanger, by Clostridium difficile toxin B, J Gen Physiol, 123(5):491-504.

Hecht, G, K Hodges, RK Gill, F Kear, S Tyagi, J Malakooti, K Ramaswamy, and PK Dudeja, 2004, Differential regulation of Na+/H+ exchange isoform activities by enteropathogenic E. coli in human intestinal epithelial cells, Am J Physiol Gastrointest Liver Physiol, 287(2):G370-378.

Heidelbaugh, JJ, M Stelwagon, SA Miller, EP Shea, and WD Chey, 2015, The spectrum of constipation-predominant irritable bowel syndrome and chronic idiopathic constipation: US

survey assessing symptoms, care seeking, and disease burden, Am J Gastroenterol, 110(4):580-587.

Hulisz, D, 2004, The burden of illness of irritable bowel syndrome: current challenges and hope for the future, J Manag Care Pharm, 10(4):299-309.

Jakab, RL, AM Collaco, and NA Ameen, 2011, Physiological relevance of cell-specific distribution patterns of CFTR, NKCC1, NBCe1, and NHE3 along the crypt-villus axis in the intestine, Am J Physiol Gastrointest Liver Physiol, 300(1):G82-98.

Janecke, AR, P Heinz-Erian, and T Muller, 2016, Congenital Sodium Diarrhea: A Form of Intractable Diarrhea, With a Link to Inflammatory Bowel Disease, J Pediatr Gastroenterol Nutr, 63(2):170-176.

Janecke, AR, P Heinz-Erian, J Yin, BS Petersen, A Franke, S Lechner, I Fuchs, S Melancon, HH Uhlig, S Travis, E Marinier, V Perisic, N Ristic, P Gerner, IW Booth, S Wedenoja, N Baumgartner, J Vodopiutz, MC Frechette-Duval, J De Lafollie, R Persad, N Warner, CM Tse, K Sud, NC Zachos, R Sarker, X Zhu, AM Muise, KP Zimmer, H Witt, H Zoller, M Donowitz, and T Muller, 2015, Reduced sodium/proton exchanger NHE3 activity causes congenital sodium diarrhea, Hum Mol Genet, 24(23):6614-6623.

Kiela, PR, D Laubitz, CB Larmonier, MT Midura-Kiela, MA Lipko, N Janikashvili, A Bai, R Thurston, and FK Ghishan, 2009, Changes in mucosal homeostasis predispose NHE3 knockout mice to increased susceptibility to DSS-induced epithelial injury, Gastroenterology, 137(3):965-975, 975 e961-910.

Kumar, A, AN Anbazhagan, H Coffing, I Chatterjee, S Priyamvada, T Gujral, S Saksena, RK Gill, WA Alrefai, A Borthakur, and PK Dudeja, 2016, Lactobacillus acidophilus counteracts inhibition of NHE3 and DRA expression and alleviates diarrheal phenotype in mice infected with Citrobacter rodentium, Am J Physiol Gastrointest Liver Physiol, 311(5):G817-G826.

Larmonier, CB, D Laubitz, FM Hill, KW Shehab, L Lipinski, MT Midura-Kiela, RM McFadden, R Ramalingam, KA Hassan, M Golebiewski, DG Besselsen, FK Ghishan, and PR Kiela, 2013, Reduced colonic microbial diversity is associated with colitis in NHE3-deficient mice, Am J Physiol Gastrointest Liver Physiol, 305(10):G667-677.

Larmonier, CB, D Laubitz, RD Thurston, AL Bucknam, FM Hill, M Midura-Kiela, R Ramalingam, PR Kiela, and FK Ghishan, 2011, NHE3 modulates the severity of colitis in IL-10-deficient mice, Am J Physiol Gastrointest Liver Physiol, 300(6):G998-G1009.

Lenzen, H, M Lunnemann, A Bleich, MP Manns, U Seidler, and A Jorns, 2012, Downregulation of the NHE3-binding PDZ-adaptor protein PDZK1 expression during cytokine-induced inflammation in interleukin-10-deficient mice, PLoS One, 7(7):e40657.

Longstreth, GF, 2007, Avoiding unnecessary surgery in irritable bowel syndrome, Gut, 56(5):608-610.

Lovell, RM and AC Ford, 2012, Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis, Clin Gastroenterol Hepatol, 10(7):712-721 e714.

McHugh, DR, CU Cotton, FJ Moss, M Vitko, DM Valerio, TJ Kelley, S Hao, A Jafri, ML Drumm, WF Boron, RC Stern, K McBennett, and CA Hodges, 2018, Linaclotide Improves Gastrointestinal Transit in Cystic Fibrosis Mice by Inhibiting Sodium/Hydrogen Exchanger 3, Am J Physiol Gastrointest Liver Physiol.

Mearin, F, BE Lacy, L Chang, WD Chey, AJ Lembo, M Simren, and R Spiller, 2016, Bowel Disorders, Gastroenterology.

Musch, MW, DL Arvans, GD Wu, and EB Chang, 2009, Functional coupling of the downregulated in adenoma Cl-/base exchanger DRA and the apical Na+/H+ exchangers NHE2 and NHE3, Am J Physiol Gastrointest Liver Physiol, 296(2):G202-210.

Rieg, T and JA Dominguez Rieg, 2017, Reply to "Reduced NHE3 activity results in congenital diarrhea and can predispose to inflammatory bowel disease", Am J Physiol Regul Integr Comp Physiol, 312(3):R312.

Saito, YA, P Schoenfeld, and GR Locke, 3rd, 2002, The epidemiology of irritable bowel syndrome in North America: a systematic review, Am J Gastroenterol, 97(8):1910-1915.

Subramanya, SB, VM Rajendran, P Srinivasan, NS Nanda Kumar, BS Ramakrishna, and HJ Binder, 2007, Differential regulation of cholera toxin-inhibited Na-H exchange isoforms by butyrate in rat ileum, Am J Physiol Gastrointest Liver Physiol, 293(4):G857-863.

Wigington, WC, WD Johnson, and A Minocha, 2005, Epidemiology of irritable bowel syndrome among African Americans as compared with whites: a population-based study, Clin Gastroenterol Hepatol, 3(7):647-653.

Woo, AL, LA Gildea, LM Tack, ML Miller, Z Spicer, DE Millhorn, FD Finkelman, DJ Hassett, and GE Shull, 2002, In vivo evidence for interferon-gamma-mediated homeostatic mechanisms in small intestine of the NHE3 Na+/H+ exchanger knockout model of congenital diarrhea, J Biol Chem, 277(50):49036-49046.

Signatures

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Nonclinical Reviewer	Dinesh Gautam	ODE III/DGIEP	Sections: 5, 16.2	**Select one:** _X_ Authored ___ Approved
	Signature: Dinesh C. Gautam -S Digitally signed by Dinesh C. Gautam -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0011025076, cn=Dinesh C. Gautam -S Date: 2019.09.10 18:25:01 -04'00'			
Nonclinical Supervisor	Sushanta Chakder	ODE III/DGIEP	Sections: 5, 16.2	**Select one:** ___ Authored _X_ Approved
	Signature: Sushanta K. Chakder -S Digitally signed by Sushanta K. Chakder -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300144003, cn=Sushanta K. Chakder -S Date: 2019.09.11 09:07:39 -04'00'			
Nonclinical Associate Director	Ronald Wange	OND IO	Sections: 5, 16.2	**Select one:** ___ Authored _X_ Approved
	Signature: Ronald L. Wange -S Digitally signed by Ronald L. Wange -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300236480, cn=Ronald L. Wange -S Date: 2019.09.11 09:35:39 -04'00'			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Clinical Pharmacology Reviewer	Sojeong Yi	OTS/OCP/DCP III	Sections: 6, 16.3	**Select one:** _X_ Authored ___ Approved
	Signature: Sojeong Yi -S Digitally signed by Sojeong Yi -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Sojeong Yi -S, 0.9.2342.19200300.100.1.1=2002075079 Date: 2019.09.09 16:21:49 -04'00'			
Clinical Pharmacology Reviewer	Hyewon Kim	OTS/OCP/DCP III	Section: 6, 16.3	**Select one:** _X_ Authored ___ Approved
	Signature: Hyewon Kim -S Digitally signed by Hyewon Kim -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Hyewon Kim -S, 0.9.2342.19200300.100.1.1=2001115262 Date: 2019.09.09 16:25:10 -04'00'			
Clinical Pharmacology Team Leader	Jie (Jack) Wang	OTS/OCP/DCP III	Section: 6, 16.3	**Select one:** ___ Authored _X_ Approved
	Signature: Jie Wang -S Digitally signed by Jie Wang -S DN: c=US o=U S Government ou=HHS ou=FDA ou=People cn=Jie Wang -S 0 9 2342 19200300 100 1 1=2000739081 Date: 2019 09 09 16:39:58 04'00'			
Clinical Pharmacology Director	Shirley Seo	OTS/OCP/DCP III	Section: 6, 16.3	**Select one:** ___ Authored _X_ Approved
	Signature: Shirley K. Seo -S Digitally signed by Shirley K. Seo -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Shirley K. Seo -S, 0.9.2342.19200300.100.1.1=1300365375 Date: 2019.09.10 10:57:50 -04'00'			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Statistical Reviewer	Shala Farr	OB/DB3	Sections: 7, 8, 16.4	**Select one:** _X_ Authored ___ Approved
	Signature: Shahla S. Farr -S Digitally signed by Shahla S Farr -S DN: c=US, o=U S Government, ou=HHS, ou=FDA, ou=People, cn=Shahla S Farr -S, 0 9 2342 19200300 100 1 1=1300090052 Date: 2019 09 10 11:15 03 04'00'			
Statistical Team Leader	George Kordzakhia	OB/DB3	Sections: 7, 8, 16.4	**Select one:** _X_ Authored _X_ Approved
	Signature: George Kordzakhia -S Digitally signed by George Kordzakhia -S DN: c=US o=U S Government ou=HHS ou=FDA ou=People 0 9 2342 19200300 100 1 1=1300390764 cn=George Kordzakhia -S Date: 2019 09 10 12:50:19 04'00'			
Division Deputy Director (OB/DBIII)	Gregory Levin		Sections: 1, 7, 8, 9, 10, 16.4	**Select one:** ___ Authored _X_ Approved
	Signature: Gregory P. Levin -S Digitally signed by Gregory P. Levin -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001127703, cn=Gregory P. Levin -S Date: 2019.09.10 19:08:38 -04'00'			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Clinical Reviewer	Elizabeth (Betsy) Mannick	ODE III/DGIEP	Sections: 1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 16.1, 16.4	**Select one:** _X_ Authored ___ Approved
	Signature: Elizabeth E. Mannick -S Digitally signed by Elizabeth E Mannick -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002526499, cn=Elizabeth E Mannick -S Date: 2019.09.11 07:57:26 -04'00'			
Clinical Team Leader	Tara Altepeter	ODE III/DGIEP	Sections: All sections approved, Authored 1.3	**Select one:** _X_ Authored _X_ Approved
	Signature: Tara Altepeter -S Digitally signed by Tara Altepeter -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Tara Altepeter -S, 0.9.2342.19200300.100.1.1=2001813963 Date: 2019.09.10 22:12:24 -04'00'			
Division Associate Director (Clinical)	Jessica J. Lee	ODE III/DGIEP	Sections: All sections approved, Authored 15	**Select one:** _X_ Authored _X_ Approved
	Signature: Jessica J. Lee -S Digitally signed by Jessica J. Lee -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Jessica J. Lee -S, 0.9.2342.19200300.100.1.1=2000596373 Date: 2019.09.11 10:33:50 -04'00'			
ODE III Director	Victor Crentsil	ODE III	Sections: All sections	**Select one:** ___ Authored _X_ Approved
	Signature: See appended signature page			

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

VICTOR CRENTSIL
09/12/2019 12:55:23 PM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	108732
Supporting document/s:	104 and 107
Applicant's letter date:	April 27, 2018; June 20, 2018
CDER stamp date:	April 27, 2018; June 20, 2018
Product:	Tenapanor (AZD1722/RDX5791)
Indication:	Irritable bowel syndrome with constipation (IBS-C)
Applicant:	Ardelyx, Inc., Fremont, CA
Review Division:	Division of Gastroenterology and Inborn Errors Products (DGIEP)
Reviewer:	Dinesh Gautam, Ph.D.
Supervisor/Team Leader:	Sushanta Chakder, Ph.D.
Division Director:	Dragos Roman, M.D. (Acting)
Project Manager:	Brian K. Strongin, R.Ph, MBA.

Category: Sodium (Na^+)/hydrogen (H^+) antiporter (NHE3) inhibitor.

Submission Content:

1. Final report of a 104-week oral carcinogenicity study of AZD1722 in rats (Study No. 3732cr) and
2. Final report of a 26-week carcinogenicity study of Tenapanor and AZ13792925 by oral gavage in CByB6F1/Tg rasH2 hemizygous mice (Study No. RDX5791-TX-16).

Background:

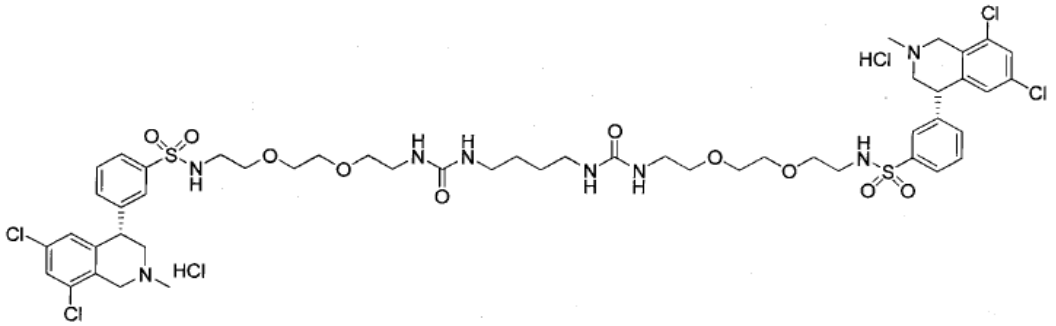
Tenapanor (AZD1722 or RDX5791), a sodium (Na^+)/hydrogen (H^+) antiporter (NHE3) inhibitor, is being developed for the treatment of constipation-related diseases such as chronic idiopathic constipation (CIC), constipation-predominant irritable bowel syndrome (IBS-C), and opioid-induced constipation (OIC). In the current submission, the Sponsor has submitted the final reports of the 2-year oral (gavage) carcinogenicity study of AZD1722 in rats and the 26-week carcinogenicity study of AZD1722, and AZ13792925 (major tenapanor metabolite) in Tg rasH2 mice.

In the 26-week carcinogenicity study in Tg rasH2 mice, tenapanor doses of 100, 300 and 800 mg/kg/day were used for females, and 10, 30 and 100 mg/kg/day for males as per the Executive

CAC recommendations. For the metabolite (AZ13792915), the doses of 55 and 165 mg/kg/day were used for both male and female mice. The control groups received the vehicle.

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET
Review of Carcinogenicity Study Results

P/T REVIEWER(s): Dinesh Gautam, Ph.D.
DATE: January 22, 2019
IND: 108,732
DRUG CODE#: RDX5791/ AZD1722
CAS#: None
DIVISION: Division of Gastroenterology and Inborn Errors Products (DGIEP)
DRUG NAME: Tenapanor
CHEMICAL STRUCTURE:



SPONSOR: Ardelyx, Inc., Fremont, CA, USA.
LABORATORY: (b) (4)
CARCINOGENICITY STUDY REPORT DATE: January 10, 2018.
THERAPEUTIC CATEGORY: For the treatment of irritable bowel syndrome with constipation (IBS-C).
PHARMACOLOGICAL CLASSIFICATION: Sodium (Na^+)/hydrogen (H^+) antiporter (NHE3) inhibitor.
MUTAGENIC/GENOTOXIC: Negative. AZD1722 was not mutagenic in the Ames bacterial reverse mutation assay, not clastogenic in an *in vitro* cultured human lymphocyte assay, and negative in the *in vivo* oral gavage mouse and intravenous rat micronucleus assays.

MICE CARCINOGENICITY STUDY

STUDY DURATION (weeks): 26
STUDY STARTING DATE: December 13, 2016
STUDY ENDING DATE: June 08, 2017
MICE STRAIN: CByB6F1/Tg rasH2
ROUTE: Oral
DOSING COMMENTS: The doses of tenapanor and AZ13792925 (a major metabolite of tenapanor) used in the 6-month oral (gavage) carcinogenicity study in CByB6F1/Tg rasH2 mice were concurred by the Executive CAC Committee. The selection of the high dose for female mice (800 mg/kg/day; 400 mg/kg BID) was based on the MFD in a 28-day oral toxicity study in CByB6F1 mice. The mid and low doses for female mice were 300 and 100 mg/kg/day (150 and 50 mg/kg BID), respectively. For male mice, the doses used were 0 (vehicle), 10, 30 and 100 mg/kg/day (0, 5, 15 and 50 mg/kg BID), the high dose being based on body weight loss in the 1-month dose range finding study.

The doses (0, 55 and 165 mg/kg/day for both male and female mice) of AZ13792925 (a major metabolite of tenapanor) used in the study was based on the maximum tolerated dose (MTD) in a 14-day and a 28-day dose range finding studies in CByB6F1 and Tg.rasH2 mice and were concurred by the Executive CAC Committee.

NUMBER OF MICE AND DOSE LEVELS:

Group No.	Test Material	Number of Animals		Dose Level (mg/kg/day)	
		Male	Female	Male	Female
1	Milli-Q Water (Negative control)	25	25	0	0
2	0.1% Tween 80 (Vehicle control)	25	25	0	0
3	1% Tween 80, 1% DMSO (Vehicle control)	25	25	0	0
4	AZ13792925 (Tenapanor metabolite)	25	25	55	55
5	AZ13792925 (Tenapanor metabolite)	25	25	165/110*	165/110*
6	Tenapanor	25	25	10	100
7	Tenapanor	25	25	30	300
8	Tenapanor	25	25	100	800
9	N-methyl-N-nitrosourea [NMU] (Positive control)	15	15	75	75
10	Health Screen/Sentinel	15	15		

*Animals were dosed at 165 mg/kg/day from Days 1 to 98. The dose level was subsequently reduced to 110 mg/kg/day beginning on Day 99 after discussion with Agency (March 14, 2017) regarding poor mean body weights.

BASIS FOR DOSES SELECTED: MFD for females; MTD for males; AZ13792925 (a major metabolite of tenapanor) doses were based on MTD.
PRIOR FDA DOSE CONCURRENCE: Yes (ExecCAC meeting minutes dated November 17, 2016, Appendix-1).
MICE CARCINOGENICITY: Negative in males and females.

MICE TUMOR FINDINGS: There were no tenapanor or AZ13792915-related neoplastic findings in male and female mice in any group. The neoplastic findings were distributed randomly among groups, and their appearance was similar to the findings in controls.

MICE STUDY COMMENTS: The doses used in the 6-month carcinogenicity study of tenapanor and AZ13792925 were concurred by the Executive CAC Committee, and the conduct of the study is appropriate for the carcinogenicity assessment of tenapanor and its major metabolite, AZ13792915.

COVERSHEET FOR CARCINOGENICITY STUDY IN MICE

1. Study No: RDX5791-TX-16
2. Name of Laboratory: (b) (4)
3. Strain: CByB6F1/Tg rasH2 hemizygous (transgenic) mice
4. No./sex/group: Negative Control (Milli-Q Water): 25
   Vehicle Control (0.1% Tween 80, 1% DMSO in Milli-Q Water): 25
   Tenapanor:
   Low dose: 25
   Mid dose: 25
   High dose: 25
   Positive Control (N-Nitrosomethylurea [NMU]): 15

   AZ13792925 (tenapanor metabolite):
   Low dose: 25
   High dose: 25
5. Dose (0, L, M, H):
   Male: Tenapanor: 0, 10, 30 and 100 mg/kg/day
   AZ13792925 0, 55 and 165 mg/kg/day
   Female: Tenapanor: 0, 100, 300 and 800 mg/kg/day
   AZ13792925 0, 55 and 165 mg/kg/day
6. Basis of dose selection stated: Yes. Based on MFD and MTD for tenapanor and AZ13792925, respectively as recommended by the Executive CAC.
7. Interim sacrifice: No
8. Total duration (weeks): 26
9. No. alive at termination:

Drug Product	Dose	**Male**		**Female**	
		Alive/total no. of animals	% survival	Alive/total no. of animals	% survival
Negative control (Milli-Q Water)	0	24/25	96	25/25	100
Vehicle control (0.1% Tween 80)	0	24/25	96	23/25	92
Vehicle control (1% Tween 80, 1% DMSO)	0	25/25	100	24/25	96
AZ13792925 (Tenapanor metabolite)	Low dose	24/25	96	25/25	100
	High dose	25/25	100	23/25	92
Tenapanor	Low dose	25/25	100	24/25	96
	Mid dose	24/25	96	24/25	96
	High dose	24/25	96	24/25	96
Positive control N-methyl-N-nitrosourea [NMU]	7.5 mg/mL (10 mL/kg)	2/15	13.33	3/15	20

10. Statistical methods used: The incidence of neoplasms and non-neoplastic findings were analyzed by using the method of Peto et. al. (Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, Pete J, Richards S, Wahrendorf J. Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In long-term and short-term screening assays for carcinogens: a critical appraisal. Annex to Supplement 2. p. 311-426. International Agency for Research on Cancer, Lyon; 1980). Prevalence analyses were used for tumors that do not lead directly or indirectly to death. Pair-wise one-sided (Peto) comparisons to the controls were also computed. For all trend and pair-wise analyses, the exact version of the Peto test was utilized. Towards positive trend, common and rare tumors were tested at p values of 0.005 and 0.025 significance level, respectively. Common and rare tumors were also compared pair-wise at p values of 0.01 and 0.05 for significance levels, respectively.

26-Week Oral Carcinogenicity Study in CByB6F1/Tg rasH2 Mice

Key study findings: Tenapanor and its metabolite (AZ13792925) was administered twice daily by oral gavage to male and female CByB6F1/Tg rasH2 mice for 26 weeks to evaluate its oncogenic potential. Male animals were administered tenapanor at dose levels of 10, 30 and 100 mg/kg/day (5, 15 and 50 mg/kg BID), and female animals were administered 100, 300 and 800 mg/kg/day (50, 150 and 400 mg/kg BID) orally. AZ13792925 (tenapanor metabolite) was administered once daily to male and female mice at dose levels of 55 and 165 mg/kg/day by oral gavage. The negative control group received Milli-Q Water (BID), the vehicle control groups received 1% Tween 80, 1% DMSO in Milli-Q Water (BID) and 0.1% Tween 80 (QD) by oral gavage. The positive control was 7.5 mg/mL N-Nitrosomethylurea (NMU) in Citrate-buffered saline (pH 4.5).

There were no tenapanor or AZ13792925-related effects on survival rates of males or females. For the positive control group, there was a high incidence of mortality in males (13 out of 15 [87%]) and females (12 out of 15 [80%]) administered NMU. Males and females in the 165 mg/kg/day AZ13792925 dose group showed reductions of body weight (13.3% and 8.3%, respectively), compared to controls (0.1% DMSO 80, in Milli-Q Water). Due to the decrease in mean body weight, the high dose of 165 mg/kg was reduced to 110 mg/kg/day on Day 99. The body weight still remained lower in males (12.8%) and females (7.0%) at study termination Day 182. No Tenapanor or AZ13792925-related neoplastic or non-neoplastic findings were noted in TgRasH2 mice. The positive control group exhibited the expected neoplastic (increased incidence of malignant lymphoma, squamous cell papilloma, and squamous cell carcinoma) changes.

Study number: RDX5791-TX-16

Volume # and page #: EDR submission, dated April 27, 2018

Conducting laboratory and location: (b) (4)

Date of study initiation: December 13, 2016

GLP compliance: A statement of compliance was included.

QA report: yes (X) no ( )

Drug, lot #, and % purity: Tenapanor Lot # X3149548 (HQ00008.5F), purity 92.2%.
AZ13792925 (metabolite), Lot # ET10834-1-P1, purity 98.1%

CAC concurrence: Yes (ExecCAC meeting minutes dated November 17, 2016, Appendix-1)

Study Type: 26-Week bioassay

Species/strain: CByB6F1/Tg rasH2 Mice

Number/sex/group; age at start of study: 8 weeks old at the initiation of dosing. Number of animals per group is provided in the Table below.

Group No.	Test Material	Number of Animals and Dose		Number of Animals and Dose	
		Male	Dose (mg/kg/day)	Female	Dose (mg/kg/day)
1	Milli-Q Water (Negative control)	25	0	25	0
2	0.1% Tween 80 (Vehicle control)	25	0	25	0
3	1% Tween 80, 1% DMSO (Vehicle control)	25	0	25	0
4	AZ13792925 (Tenapanor metabolite)	25	55	25	55
5	AZ13792925 (Tenapanor metabolite)	25	165/110*	25	165/110*
6	Tenapanor	25	10	25	100
7	Tenapanor	25	30	25	300
8	Tenapanor	25	100	25	800
9	N-methyl-N-nitrosourea [NMU] (Positive control)	15	75	15	75
10	Health Screen/Sentinel	15		15	

*Animals were dosed at 165 mg/kg/day from Days 1 to 98. The dose level was subsequently reduced to 110 mg/kg/day beginning on Day 99 after discussion with Agency (March 14, 2017) regarding poor mean body weights.

Animal housing: Animals were housed individually in polycarbonate cages equipped with an automatic watering valve.

Formulation/vehicle:

- Tenapanor and AZ13792925 dosing formulations were prepared in 1% Tween 80, 1% DMSO in Milli-Q Water. The positive control article (N-methyl-N-nitrosourea [NMU]) was prepared fresh in citrate-buffered saline on the day of dosing (Day 3).

Drug stability/homogeneity: Formulations samples from the top, middle and bottom of the container were assessed for homogeneity, stability and concentration on Weeks 1, 4, 12, 16, 20, and 26. The samples were found to be stable at room temperature.

Methods:

Doses: Male: Tenapanor: 0, 10, 30 and 100 mg/kg/day
AZ13792925 0, 55 and 165 mg/kg/day
Female: Tenapanor: 0, 100, 300 and 800 mg/kg/day
AZ13792925 0, 55 and 165 mg/kg/day

Basis of dose selection: Based on MFD and MTD for tenapanor and AZ13792925, respectively as recommended by the Executive CAC.

Restriction paradigm for dietary restriction studies: None

Route of administration: Oral gavage.

Frequency of drug administration: Tenapanor: Twice daily
AZ13792925: Once daily

Dual controls employed: Yes (0.1% Tween 80 and 1% Tween 80, 1% DMSO)

Interim sacrifices: None

Study Design: The study design is shown in the table below (from page 28 and 29 of the study report).

Text Table 6
Experimental Design - Carcinogenicity

Group No.	Test Material	Dose Level (mg/kg/day)		Dose Volume (mL/kg)	Adjusted Dose Concentration (mg/mL)[e]		Number of Animals	
							Carcinogenicity Study	
		Male	Female		Male	Female	Male	Female
1	Milli-Q Water	0[a]	0[b]	10	0	0	25	25
2	0.1% Tween 80	0[c]	0[c]	10	0	0	25	25
3	1% Tween 80, 1% DMSO	0[a]	0[b]	10	0	0	25	25
4	AZ13792925	55[c]	55[c]	10	5.5	5.5	25	25
5	AZ13792925	165/ 110[c f]	165/ 110[c f]	10	16.5/ 11[f]	16.5/ 11[f]	25	25
6	Tenapanor	10[a]	100[b]	10	0.54	5.4	25	25
7	Tenapanor	30[a]	300[b]	10	1.62	16.2	25	25
8	Tenapanor	100[a]	800[b]	10	5.4	43.2	25	25
9	NMU (Positive Control)	75[d]	75[d]	10	7.5	7.5	15	15
10	Health Screen/Sentinel	-	-	-	-	-	15	15

- = not applicable.
[a] Dosed twice/day at 0, 5, 15, or 50 mg/kg/dose (0, 10, 30, or 100 mg/kg/day) approximately 6 hours ± 1 hour apart.
[b] Dosed twice/day at 0, 50, 150, or 400 mg/kg/dose (0, 100, 300, or 800 mg/kg/day) approximately 6 hours ± 1 hour apart.
[c] Dosed once/day.
[d] Dosed only once on Day 3 via intraperitoneal injection.
[e] Adjusted concentrations for Tenapanor only based on a correction factor of 1.08.
[f] Animals were dosed at 165 mg/kg/day from Days 1 to 98. The dose level was subsequently reduced to 110 mg/kg/day beginning on Day 99 after discussion with FDA regarding poor mean body weights.

Satellite group for toxicokinetics: Yes.

Deviations from original study protocol: There were minor protocol deviations which did not have any impact on the results and study interpretations.

Statistical methods:

Mortality: Kaplan-Meier estimates of group survival rates were calculated. Mortality data were analyzed by comparing different dosage groups by using nonparametric time-adjusted methods, for example, log-rank, including a two-sided test for trend with dose and pair-wise two-sided comparisons to controls.

Tumor Data: Statistical analysis of the tumor incidence data was conducted in accordance with the FDA draft *Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals*. 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 and Fisher's exact test were used to compare the statistical significance of the treatment groups with the control group. The survival adjusted test was conducted according to the prevalence/mortality methods. The fatal and incidental tumors were analyzed by survival adjusted trend test (Peto et al., 1980). Each diagnosed tumor type was analyzed separately and, analysis of combined tumor types were performed as described by McConnell et al (J Natl Cancer Inst 1986:76:283-289). Evaluation criteria (p-values of significance) were applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%).

Observations and times:

Mortality: Twice daily, once in the morning and once in the evening.

Clinical Signs: Cage side clinical observation was performed once daily. Detailed clinical observation was performed once weekly.

Body weights: Once weekly.

Food consumption: Food consumption was measured weekly.

Ophthalmoscopy: Not conducted.

Hematology: Blood samples were collected to analyze the hematological parameters.

Gross pathology: At necropsy

Organ Weights: Following organs were weighed at necropsy for all scheduled euthanasia animals (from page 36 of the study report).

Text Table 15
Organs Weighed at Necropsy

Brain	Liver
Epididymis[a]	Lung
Gland, adrenal[a]	Ovary[a]
Gland, pituitary[b]	Spleen
Gland, prostate	Testis[a]
Gland, thyroid[a, b]	Thymus
Heart	Uterus
Kidney[a]	

[a] Paired organ weight.
[b] Weights were collected post fixation.

Histopathology: The following tissues from all main study animals were examined.

Text Table 16
Tissue Collection and Preservation

Animal identification	Large intestine, cecum
Artery, aorta	Large intestine, colon
Body cavity, nasal	Large intestine, rectum
Bone marrow smear	Larynx
Bone marrow	Liver
Bone, femur	Lung
Bone, sternum	Lymph node, mandibular
Brain	Lymph node, mesenteric
Cervix	Muscle, skeletal
Epididymis	Nerve, optic[a]
Esophagus	Nerve, sciatic
Eye[a]	Ovary
Gallbladder	Oviduct
Gland, adrenal	Pancreas
Gland, harderian	Skin
Gland, lacrimal	Small intestine, duodenum
Gland, mammary	Small intestine, ileum
Gland, parathyroid	Small intestine, jejunum
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary	Stomach
Gland, seminal vesicle	Testis[b]
Gland, thyroid	Thymus
Gland, zymbal	Tongue
Gross lesions/masses	Trachea
Gut-associated lymphoid tissue	Urinary bladder
Heart	Uterus
Kidney	Vagina

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

Toxicokinetics: Blood samples from TK animals were collected on Day1 and Day 180. The Sponsor's Tables below show the TK sample collection schedule (from page 32 of the study report).

Text Table 10
TK Sample Collection Schedule – Day 1

Group No.	Subgroup	No. of Males/ Females	Sample Collection Time Points (Time Post First Daily Dose) on Day 1					
			0.5 hr	1 hr	2 hr	4 hr	8 hr	24 hr
2 and 3	A	3/3	X	-	-	-	-	-
	B	3/3	-	X	-	-	-	-
4, 5, 6, 7, and 8	A	3/3	X	-	-	-	-	-
	B	3/3	-	X	-	-	-	-
	C	3/3	-	-	X	-	-	-
	D	3/3	-	-	-	X	-	
	E	3/3	-	-	-	-	X	-
	F	3/3	-	-	-	-	-	X

X = sample collected; - = not applicable.

Text Table 11
TK Sample Collection Schedule – Day 180

Group No.	Subgroup	No. of Males/ Females	Sample Collection Time Points (Time Post First Daily Dose) on Day 180					
			0.5 hr	1 hr	2 hr	4 hr	8 hr	24 hr
2 and 3	G	3/3	X	-	-	-	-	-
	H	3/3	-	X	-	-	-	-
4, 5, 6, 7, and 8	G	3/3	X	-	-	-	-	-
	H	3/3	-	X	-	-	-	-
	I	3/3	-	-	X	-	-	-
	J	3/3	-	-	-	X	-	
	K	3/3	-	-	-	-	X	-
	L	3/3	-	-	-	-	-	X

X = sample collected; - = not applicable.

Results:

Mortality: There was no tenapanor- and AZ13792925-related effect on survival. The survival rates were similar among all treated groups and both sexes. Summary of the survival data and Kaplan-Meier survival plots for male and female mice are provided below.

Table: Survival rate of male and female mice

Sex	Group	Test Material	Dose (mg/kg/day)	No. of Mice	No of Deaths	No. of survivors at Termination	Survival %
Male	1	Milli-Q Water	0	25	1	24	96
	2	0.1% Tween 80	0	25	0	25	100
	3	1% Tween 80 & 1% DMSO	0	25	1	24	96
	4	AZ13792925	55	25	1	24	96
	5	AZ13792925	165/110**	25	0	25	100
	6	Tenapanor	10	25	0	25	100
	7	Tenapanor	30	25	1	24	96
	8	Tenapanor	100	25	1	24	96
	9	NMU*	75	15	13	2	13
Female	1	Milli-Q Water	0	25	0	25	100
	2	0.1% Tween 80	0	25	1	24	96
	3	1% Tween 80 & 1% DMSO	0	25	2	23	92
	4	AZ13792925	55	25	0	25	100
	5	AZ13792925	165/110*	25	2	23	92
	6	Tenapanor	100	25	1	24	96
	7	Tenapanor	300	25	1	24	96
	8	Tenapanor	800	25	1	24	96
	9	NMU*	75	15	12	3	20

*NMU = N-Nitrosomethylurea in citrate-buffered saline (Positive Control)
**Dose level was reduced to 110 mg/kg/day on Day 99

Figure 2A: Kaplan-Meier Survival Curves for
Male Mice Tenapanor

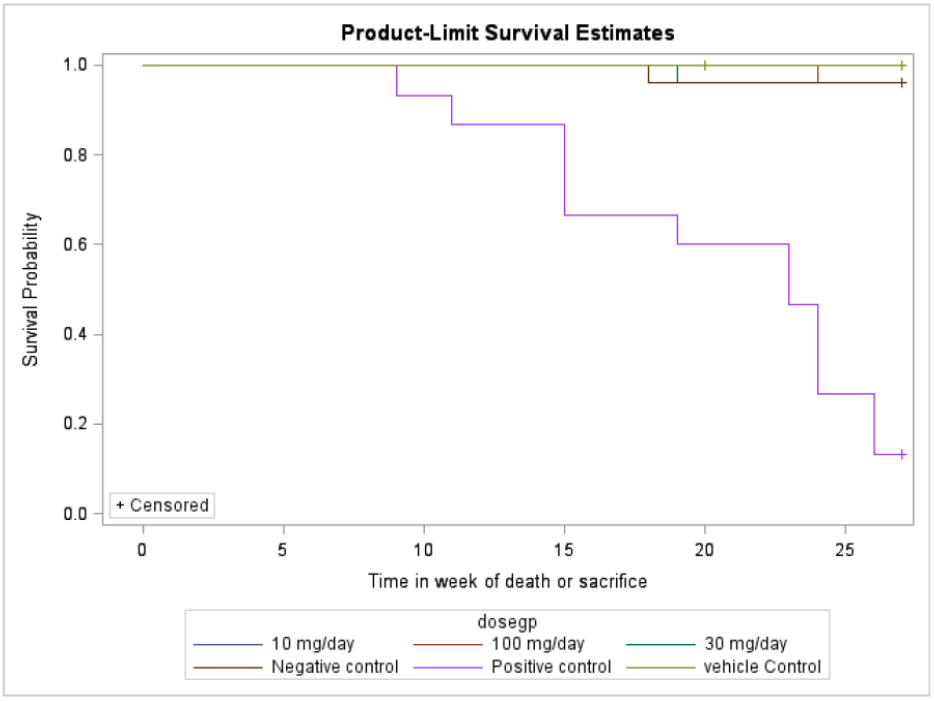


Figure 2B: Kaplan-Meier Survival Curves for
Female Mice Tenapanor

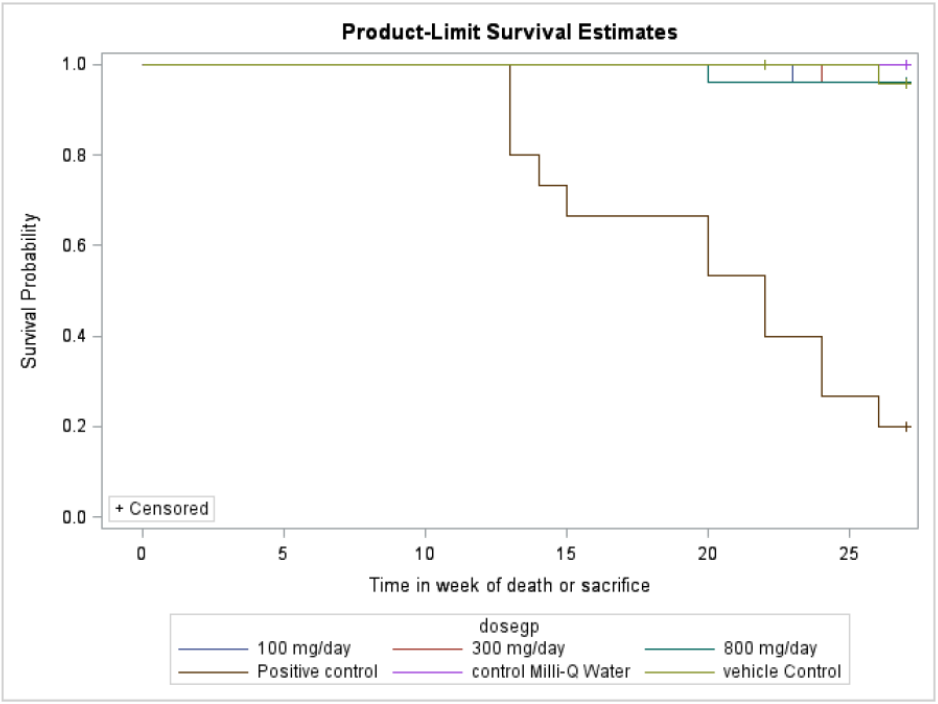


Figure 2C: Kaplan-Meier Survival Curves for
Male Mice AZ13792925

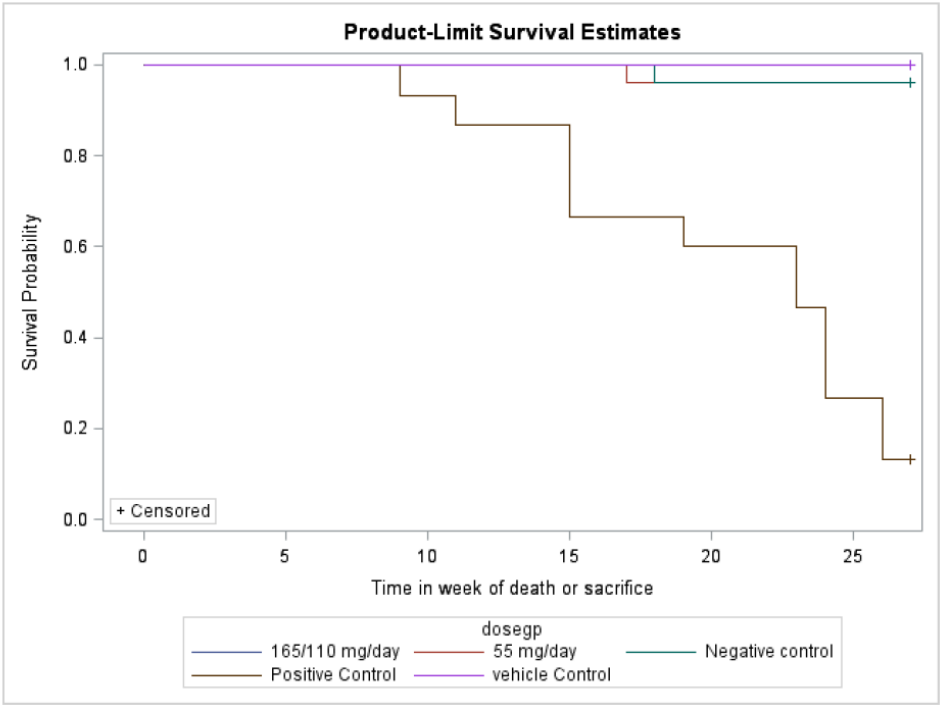
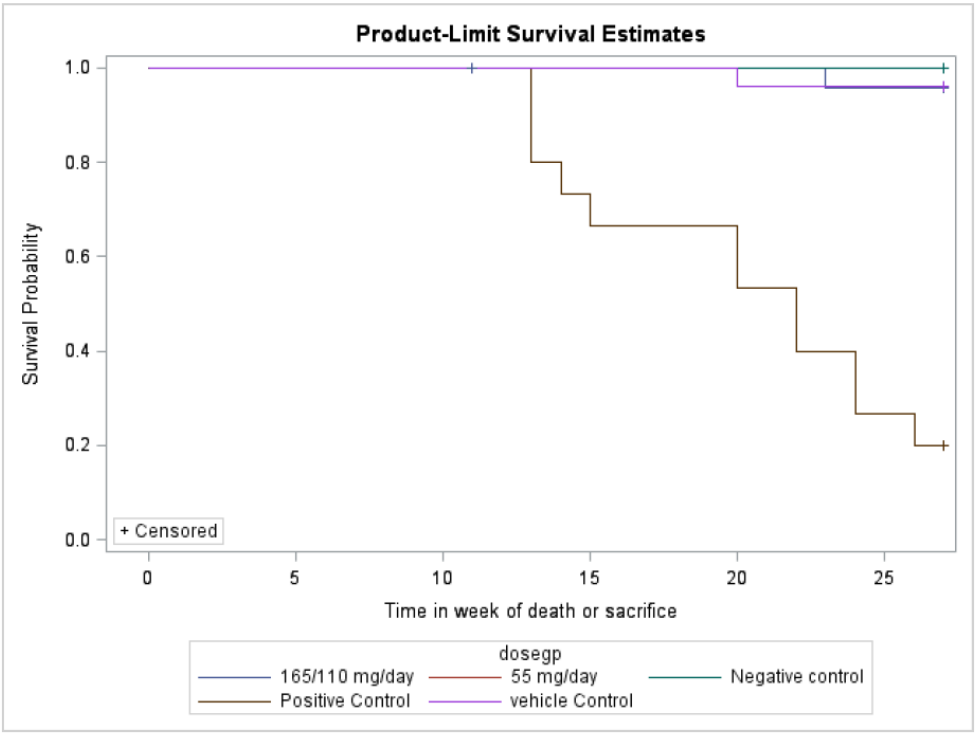


Figure 2D: Kaplan-Meier Survival Curves for
Female Mice AZ13792925



Clinical signs: No tenapanor- and AZ13792925-related clinical signs were observed. However, females administered 165/110 mg/kg/day AZ13792925 exhibited dehydration, hunched posture, and thin appearance. Clinical signs in the NMU (positive control) mice included decreased activity, abdominal distension, cold to touch, dehydration, hunched posture, thin appearance, high incidence of skin papules, ocular discharge, ungroomed, lying on side, and changes in respiration (e.g., increased, irregular, or shallow).

Body weight: There was no significant effect of treatment on the body weight of males and females following treatment of tenapanor for 26 weeks. Mean body weights were lower for AZ13792925 165 mg/kg/day males (12.5% on Day 92) and females (9.1% on Day 50) prior to the reduction in dose level on Day 99 to 110 mg/kg/day, compared to their 01% Tween 80 controls. Following reduction of dose to 110 mg/kg/day, males showed 12.8% and females showed 7.0% reduction in mean body weight at study termination Day 182. Mean body weights of mice administered NMU (positive control) were significantly lower than controls.

Food consumption: There was no test article-related effect on food consumption. However, mean food consumption in the AZ13792925 165/110 mg/kg/day males and females was generally lower than the 0.1% Tween controls beginning on Days 8 to 15 (male: 4.99g vs 6.72g; female 5.20g vs 7.40g).

Hematology: There were no test article-related changes in hematology parameters during the study.

Organ Weights: At ≥300 mg/kg tenapanor, absolute and relative (to body weight) weights of the uterus and spleen were lower in females, compared to control. In males, the absolute and relative weights of the adrenal glands were increased at ≥30 mg/kg tenapanor, compared to control. In the positive control female group, increased absolute and relative weights of the liver, kidneys, spleen, and uterus were due to malignant lymphoma.

Female	**Uterus**		**Spleen**		**Male**	**Adrenal gland**	
Tenapanor (mg/kg/day)	Absolute weight (g)	Relative weight (%)	Absolute weight (g)	Relative weight (%)	**Tenapanor (mg/kg/day)**	Absolute weight (g)	Relative weight (%)
0	0.3146	1.39578	0.1009	0.42610	**0**	0.00454	0.01522
100	0.2752	1.10472*	0.0917	0.36785	**10**	0.00490	0.01647
300	0.2130*	0.88612*	0.0764*	0.31908*	**30**	0.00526*	.001774*
800	0.1797*	0.77241*	0.0772*	0.33254*	**100**	0.00545*	0.01962*

*p=0.05-0.001

Gross pathology: There were no AZ13792925- or tenapanor-related gross pathology findings at unscheduled or scheduled necropsies. The findings were distributed randomly among groups.

Histopathology:

Non-neoplastic**:** There were no AZ13792925- or Tenapanor-related non-neoplastic observations. Non-neoplastic findings were distributed randomly among groups, or their appearance was similar to findings in controls. NMU-administered positive control mice exhibited expected non-neoplastic

histopathology changes in the retina (degeneration affecting the outer nuclear layer in 100% of males and females examined).

Neoplastic**:** There were no statistically significant findings for tumor incidences among males or females. Neoplastic findings noted among mice administered control articles (Milli-Q water, 0.1% Tween 80, or 1% Tween 80 & 1% DMSO) or test articles (AZ13792925 or Tenapanor) occurred sporadically with a low incidence rate that was non dose-responsive. The neoplastic lesions for different groups are presented in Appendix 3 (from page 1468-1493 of the study report).

The data were also reviewed by the FDA statistician (Malick Mbodj, PhD), and did not show a significant increase in the neoplastic incidences for individual organs or after combining the tumor incidences related to test articles (AZ13792925 or Tenapanor). As expected, for the positive control group, there was a high incidence of mortality in males (13 out of 15 = 87%) and females (12 out of 15 = 80%) administered NMU. The positive control group exhibited neoplastic (increased incidence of malignant lymphoma, squamous cell papilloma, and squamous cell carcinoma) and non-neoplastic (retinal degeneration) changes, thereby validating the CByB6F1-Tg-(HRAS)2Jic hemizygous mouse model used in this study.

Toxicokinetics: Peak plasma concentrations of AZ13792925 were observed within 0.5 hours. The half-life ($t_{1/2}$) of AZ13792925 ranged from 1.75 to 4.24 hours in males and 1.67 to 6.40 hours in females. The C_{max} and $AUC_{(0-24h)}$ were increased with an increase in dose in a greater than dose-proportional manner in males and females on Day 1 and Day 180. There was a trend towards increased AZ13792925 exposure in terms of AUC_{0-24} in female mice when compared to males with fold changes that ranged from 2- to 3-fold higher. Accumulation ratios were 1.86 and 1.31 at the 55 mg/kg/day dose level in males and females, respectively. The TK parameters of AZ13792925 for male and female animals are shown in the Table below (from page 47 of the report).

Toxicokinetic Parameters for AZ13792925 Following AZ13792925 Administration Once Daily in Mice Group 4 and Group 5

Gender:	**Males**		**Females**	
AZ13792925 Dosage (mg/kg/day):	**55**	**165/110[a]**	**55**	**165/110 [a]**
Parameter (Units)	Day 1			
AUC_{0-24} (ng·h/mL)	16,700	144,000	45,200	290,000
AUC_{inf} (ng·h/mL)	16,700	146,000	45,200	310,000
Cmax (ng/mL)	7,100	22,800	12,600	40,400
Tmax (h)	0.5	0.5	0.5	0.5
T1/2 (h)	1.75	4.24	1.67	6.40
	Day 180			
AUC_{0-24} (ng·h/mL)	31,200	89,500	59,100	197,000
AUC_{inf} (ng·h/mL)	31,200	89,500	59,100	197,000
C_{max} (ng/mL)	11,100	26,600	15,500	31,200
T_{max} (h)	0.5	0.5	0.5	0.5
T1/2 (h)	1.88	1.88	1.99	1.74
Accumulation Ratio	1.86	NC	1.31	NC

NC = Not calculable due to change in dose during treatment period.
[a] Animals were dosed at 165 mg/kg/day from Day 1 to Day 98 and at 110 mg/kg/day beginning on Day 99.

After oral administration of tenapanor to male and female mice, peak plasma concentrations of the metabolite, AZ13792925 were observed within 0.5 to 1 hour following the first dose. The half-life ($T_{1/2}$) of AZ13792925 was not calculated. The C_{max} and $AUC_{(0\text{-inf})}$ of AZ13792925 increased with increasing dose in a less than dose-proportional manner on both evaluation days. Accumulation ratios were 1.72 and 2.78 at the 100 mg/kg/day dose level in males and 800 mg/kg/day in females, respectively. The TK parameters of AZ13792925 following oral administration of tenapanor to male and female mice are shown in the Table below (from page 48 of the report).

Text Table 24
Toxicokinetic Parameters for AZ13792925 Following Tenapanor Administration Twice Daily in Mice in Group 6, Group 7, and Group 8

Gender:	**Males**			**Females**		
Tenapanor Dosage (mg/kg/day):	**10**	**30**	**100**	**100**	**300**	**800**
Parameter (Units)	Day 1					
$AUC_{0\text{-}6}$ (ng·h/mL)	1.11	3.60	5.74	11.9	16.3	23.5
AUC_{last} (ng·h/mL)	1.39	4.13	7.00	39.9	68.7	80.0
C_{max} (ng/mL)	1.17	1.70	2.57	4.57	5.36	6.27
T_{max} (h)	0.5	0.5	0.5	0.5	0.5	1
Tlast (h)	8	8	8	24	24	24
	Day 180					
$AUC_{0\text{-}6}$ (ng·h/mL)	1.79	5.04	9.85	13.7	27.9	65.2
AUC_{last} (ng·h/mL)	2.79	7.34	14.8	20.9	35.5	212
C_{max} (ng/mL)	0.913	1.61	3.85	4.41	8.83	14.4
T_{max} (h)	1	0.5	0.5	8	1	0.5
T_{last} (h)	8	8	8	8	8	24
Accumulation Ratio	1.61	1.40	1.72	1.15	1.72	2.78

Note: T_{max} is presented as time from first dose; AUC_{inf} and $T_{1/2}$ were not reported as the majority of values being not reportable due to $\%AUC_{Extrap} > 20\%$ and/or $r^2 < 0.80$ or not calculable due to limited data in the terminal elimination phase.

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The study methodology appears to be appropriate and acceptable.

Evaluation of tumor findings: There was no tenapanor- and AZ13792925-related increase in tumor incidences in male and female Tg rasH2 mice.

RATS CARCINOGENICITY STUDY

STUDY DURATION (weeks): 104
STUDY STARTING DATE: January 20, 2014
STUDY ENDING DATE: 18-29 January, 2016
RAT STRAIN: Crl:CD(SD) rats
ROUTE: Oral (gavage)
DOSING COMMENTS: The tenapanor dose levels used in the 2-year rat carcinogenicity study were 0 (vehicle), 1, 3, and 10 mg/kg/day. The high-dose of 10 mg/kg/day was selected based on the MTD from the 14-day and 6-month toxicology studies in rats, and was concurred by the CDER Exec CAC (Exec CAC meeting minutes dated December 17, 2013, Appendix 3). Beginning on Day 69, due to extended clinical signs and body weight loss, both male and female rats in the 10 mg/kg/day (high) dose group were placed on a dosing holiday. On April 11, 2014 (SDN 034), the Sponsor submitted a protocol amendment for the reduction of the high dose from 10 mg/kg/day to 5 mg/kg/day based on body weight loss (up to 20% at the mid and high doses during Week 8 of the study), and the ECAC concurred with the Sponsor's proposal for the dose reduction. In addition to agreeing with the reduction of the high dose, the ECAC also recommended that the mid-dose be reduced from 3 mg/kg/day to 2 mg/kg/day. On Day 86, the 10 mg/kg/day (high) dose level was reduced to 5 mg/kg/day due to decrease in body weight up to 20%. On Day 92, for both male and female rats, the 3 mg/kg/day (mid) dose level was reduced to 2 mg/kg/day.

Males in group 4 (10/5 mg/kg/day) were terminated early during Week 83, as only 15 animals survived in the group. All other male groups were terminated during Week 86 after only 20 animals survived in the male vehicle control group. All female groups were terminated on Week 96 due to survival in the vehicle control female group reached 20 animals.

NUMBER OF RATS:

Study Group	**Number of Animals**	
	Male	**Female**
Control (C1)	60	60
Low Dose	60	60
Middle Dose	60	60
High Dose	60	60

RATS DOSE LEVELS:

Study Group	**Dose Level (mg/kg/day)**
Control (C1)	0
Low Dose	1
Middle Dose	3/2*
High Dose	10/5**

* On Day 92, 3 mg/kg/day dose was reduced to 2 mg/kg/day
** On Day 86, 10 mg/kg/day dose was reduced to 5 mg/kg/day.

BASIS FOR DOSES SELECTED: Maximum Tolerated Dose (MTD).

PRIOR FDA DOSE CONCURRENCE: Yes (Exec CAC meeting minutes dated December 17, 2013, Appendix 3).
RAT CARCINOGENICITY: Negative in male and female rats.
RAT TUMOR FINDINGS: Once daily oral gavage administration of AZD1722 at dose levels of 0, 1, 3/2, or 10/5 mg/kg/day for males up to Week 86 and for females up to Week 96 did not result in an increase in tumor incidences.

RAT STUDY COMMENTS: The high-dose selection was based on the MTD, and was concurred by the Executive CAC. The study appears to be valid and acceptable.

COVERSHEET FOR CARCINOGENICITY STUDY IN RATS

1. Study No: 3732(b) (4)
2. Name of Laboratory: (b) (4)
3. Strain: Crl:CD®(SD) rats
4. No./sex/group: 60 animals/sex/group.
5. Dose (0, L, M, H): 0, 1, 3/2 and 10/5 mg/kg/day for both males and females.
6. Basis of dose selection stated: Based on MTD, and was concurred with by the Executive CAC.
7. Interim sacrifice: No
8. Total duration (weeks): Males: 86 weeks; Females: 96 weeks
9. No. alive at termination:

	Male		Female	
	Alive/total no. of animals	% survival	Alive/total no. of animals	% survival
Control	20/60	33	20/60	33
Low dose	15/60	25	22/60	37
Mid dose	22/60	37	14/60	23
High dose	15/60	25	17/60	28

10. Statistical methods used: The incidence of neoplastic and non-neoplastic findings were analyzed by time-adjusted Peto et. al. methods (Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, Pete J, Richards S, Wahrendorf J. Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In long-term and short-term screening assays for carcinogens: a critical appraisal. Annex to Supplement 2. p. 311-426. International Agency for Research on Cancer, Lyon; 1980). Prevalence analyses were used for tumors that did not lead directly or indirectly to death. Pair-wise one-sided (Peto) comparisons to the controls were also computed. For all trend and pair-wise analyses, the exact version of the Peto test was utilized. Towards positive trend, common and rare tumors were tested at p values of 0.005 and 0.025 significance levels, respectively. Common and rare tumors were also compared pair-wise at p values of 0.01 and 0.05 significance levels, respectively.

104-Week Oral Carcinogenicity Study in Crl:CD1®(SD) Rat:

Key study findings: Tenapanor (AZD1722) was administered once daily by oral gavage at dose levels of 0, 1, 3/2 and 10/5 mg/kg/day to male and female Crl:CD rats for 83 to 96 weeks. The high-dose selection was based on the MTD, and was concurred by the Exec CAC. Due to body weight loss in mid and high dose groups, the high dose was reduced from 10 mg/kg/day to 5 mg/kg/day, and the mid- dose was reduced from 3 mg/kg/day to 2 mg/kg/day, and was concurred by the Exec CAC. The high and mid dose levels were adjusted on Days 86 (Week 13) and 92 (Week 14), respectively.

There were no tenapanor- related statistically significant findings among males or females for survival rates. Tenapanor treated animals showed up to 20% reduction in body weight within the first 3 months of the study, which prompted dose level reductions. Gross pathology (dark, green, mucoid, watery, gritty, and/or soft material) and microscopic (ulceration, mixed cell inflammation) findings in the gastrointestinal tract were generally most pronounced in the cecum, and there were higher incidences in males at ≥1 mg/kg/day and females at ≥3/2 mg/kg/day.
There was no significant positive trend for any tumor in male or female rats. Thus, tenapanor did not cause any treatment-related neoplasms in rats in the 2-year oral carcinogenicity study.

Study number: 3732(b) (4)

Volume # and page #: EDR submission

Conducting laboratory and location: (b) (4)

Date of study initiation: January 20, 2014

GLP compliance: A statement of compliance was included.

QA report: yes (X) no ( )

Drug, lot #, and % purity: AZD1722, Lot # 13-001538AZ, purity 99.8%.

CAC concurrence: Yes, the doses used in the 2-year rat carcinogenicity study was concurred with by the Exec CAC (ExecCAC meeting minutes dated December 17, 2013, Appendix 3)

Study Type: 2-year bioassay

Species/strain: Crl:CD1®(SD) Rat

Number/sex/group; age at start of study: 8 weeks old at the time of study. Number of animals per group is given in the Table below.

Study Group	Number of Animals	
	Male	Female

Control (C1)	60	60
Low Dose	60	60
Middle Dose	60	60
High Dose	60	60

Animal housing: Animals were housed individually in stainless steel wire mesh-bottomed cages equipped with an automatic watering valve. The targeted conditions for animal room environment and photoperiod were as follows: Temperature: 68-79°F, Humidity: 30 - 70%, Light cycle: 12 hours light and 12 hours dark cycle.

Formulation/vehicle: 0.1% (v/v) TWEEN 80 in Milli-Q Water.

Drug stability/homogeneity: Samples of prepared formulations mixed for the homogeneity analysis were collected from the middle of the container. Concentration results were considered acceptable if the mean sample concentrations were within or equal to ± 10% of the theoretical concentration. The dosing formulation was found to be homogeneous and stable for at least 1 day when stored at ambient laboratory temperature in the dark and at least 8 days when stored at 2 to 8^0C in the dark.

Methods:

Doses: 0, 1, 3/2, and 10/5 mg/kg/day

Basis of dose selection: Based on MTD, and the doses were concurred with by the Executive CAC on June 2, 2005.

Restriction paradigm for dietary restriction studies: Not applicable

Route of administration: Oral gavage.

Frequency of drug administration: Once daily

Dual controls employed: No

Interim sacrifices: None

Study Design: The study design is shown in the Table below (from page 15 of the study report).

Experimental Design

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals			
					Carcinogenicity Study		Toxicokinetic Phase	
					Males	Females	Males	Females
1	Control Article[a]	0	5	0	60	60	3	3
2	AZD1722	1	5	0.2	60	60	3	3
3	AZD1722	3/2[b]	5	0.6/0.4[b]	60	60	3	3
4	AZD1722	10/5[c]	5	2/1[c]	60	60	3	3

[a] 0.1% (v/v) TWEEN® 80 in Milli-Q water.
[b] The dose level for Group 3 was decreased to 2 mg/kg/day beginning during Week 14 (Day 92) on 21 Apr 2014.
[c] The dose level for Group 4 was decreased to 5 mg/kg/day beginning during Week 13 (Day 86) on 15 Apr 2014.

Satellite group for toxicokinetics: Yes (shown in the above Table).

Deviations from original study protocol: There were minor protocol deviations which did not have any impact on the results and study interpretations.

Statistical methods:

Mortality: The Kaplan-Meier's curves were presented graphically for male and female rats separately. Mortality data were analyzed by comparing different dosage groups by using nonparametric time-adjusted methods, for example, log-rank, including a two-sided test for trend with dose and pair-wise two-sided comparisons to controls.

Tumor Data: The incidence of neoplastic and non-neoplastic findings were analyzed by time-adjusted Peto et. al. methods using cause of death information for the treatment groups. Prevalence analyses were used for tumors that did not lead directly or indirectly to death; otherwise, life table methods were used (for fatal and palpable tumors). Pair-wise one-sided (Peto) comparisons to the controls were also computed. For all trend and pair-wise analyses, the exact version of the Peto test was utilized.

Observations and times:

Mortality: Twice daily, once in the morning and afternoon throughout the study.

Clinical Signs: Twice daily. Cage side clinical observations were performed once daily beginning during Week -1 and continuing throughout the dosing phase. A detailed clinical examination was performed once during each study week.

Body weights: Each animal was weighed at least once weekly, starting Week -1, twice weekly for Weeks 1 to 4, once weekly for Weeks 5 to 9, twice weekly for Weeks 10 to 18, once weekly for Weeks 19 to 24, once every 2 weeks for Weeks 26 to 36, and once every 4 weeks thereafter.

Food consumption: Food consumption was measured and recorded once weekly, starting on Day -7, through Week 14, once every 2 weeks for Weeks 16 to 26, and once every 4 weeks thereafter.

Ophthalmoscopy: Ophthalmological examinations were performed on Day -9 and during Weeks 52 and 83/86/95.

Hematology: Blood samples were collected at gross necropsy for determination of the hematology parameters.

Serum Chemistry: Blood samples were collected to determine serum sodium levels in the toxicokinetic animals prior to termination during Week 52.

Serology: Serological tests were performed at Weeks 26, 52, 78, 88 (males only), and 96 (females only) to determine the following diseases: pneumonia virus of mice (PVM), reovirus type 3 (REO), mouse encephalomyelitis virus (GDVII), Sendai virus (SEND), lymphocytic choriomeningitis virus (LCMV), Kilham rat virus (KRV), rat coronavirus/sialodacryoadenitis virus (SDAV), *Mycoplasma pulmonis* (MPUL), and Toolan's H-1 virus (H-1). At the 12- and 18-month evaluation periods, serological evidence of the following additional parameters was determined: non-structural protein 1 (NS-1), rat parvovirus (RPV), epizootic diarrhea of infant mice (EDIM), cilia-associated respiratory bacillus (CARB), and rat minute virus (RMV). Additionally, perianal cellophane tape tests for pinworm eggs and floatation method tests on feces for parasite eggs were conducted.

Gross pathology: At necropsy

Organ Weights: Not measured.

Histopathology: All tissues from all main study animals were examined. The tissues examined for the main study groups are shown in the following list (from page 33 of the study report).

Text Table 14
Tissue Collection and Preservation

Animal identification	Large intestine, cecum
Artery, aorta	Large intestine, colon
Bone marrow smear[a]	Large intestine, rectum
Bone marrow, femur	Liver
Bone marrow, sternum	Lung
Bone, femur	Lymph node, mandibular
Bone, sternum	Lymph node, mesenteric
Brain	Muscle, skeletal
Cervix	Nerve, optic[b]
Epididymis	Nerve, sciatic
Esophagus	Ovary
Eye[b]	Oviduct
Gland, adrenal	Pancreas
Gland, harderian	Skin
Gland, lacrimal	Small intestine, duodenum
Gland, mammary	Small intestine, ileum
Gland, parathyroid	Small intestine, jejunum
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary mandibular/sublingual	Stomach
Gland, salivary parotid	Testis[c]
Gland, seminal vesicle	Thymus
Gland, thyroid	Tongue
Gland, zymbal	Trachea
Gross lesions/masses	Ureter
Gut-associated lymphoid tissue	Urinary bladder
Heart	Uterus
Kidney	Vagina

[a] Collected at scheduled and unscheduled necropsies; not collected from animals found dead or euthanized moribund and then stored in the refrigerator prior to necropsy.
[b] Preserved in Davidson's fixative.
[c] Preserved in Modified Davidson's fixative.

Toxicokinetics: Blood samples were collected from the treatment group animals at schedules shown in the Sponsor's Table below (from page 30 of the study report).

Text Table 12
Toxicokinetic Sample Collection Schedules

Group No(s).	Sample Collection Time Points (Time Postdose) at the end of Weeks 4 and 26	
	4 hr	24 hr
1	X	X
2 to 4	X	X

X = sample collected.

Group No(s).	Sample Collection Time Points (Time Postdose) Week 52					
	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
1	-	-	X	-	-	X
2 to 4	X	X	X	X	X	X

X = sample collected; - = not applicable.

Results:

Mortality: There was no tenapanor-related effect on mortality. The survival rates were similar among all groups and both sexes. A summary of the survival data and Kaplan-Meier survival plots are presented below (page 14, 22 and 23 from statistical review by Malick Mbodj, Ph.D.).

Table1A: Intercurrent Mortality Rate
Male Rats

	0 mg|kg|day		1 mg|kg|day		3/2# mg|kg|day		10/5# mg|kg|day	
Week	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 50	9	15.00	13	21.67	10	16.67	14	23.33
51 - 80	26	58.33	27	66.67	25	58.33	29	71.67
81 - 86	5	66.67	5	75.00	3	63.33	2	75.00
Ter. Sac.	19	31.67	15	25.00	21	35.00	15&	25.00
Total	60	100.00	60	100.00	60	100.00	60	100.00

#: The dose level for Group 4 was decreased to 5 mg/kg/day beginning Week 13 (Day 86), and dose level for Group 3 was decreased to 2 mg/kg/day beginning Week 14 (Day 92).
&: Group 4 males terminated early during Week 83

Table1B: Intercurrent Mortality Rate
Female Rats

	0 mg|kg|day		1 mg|kg|day		3/2# mg|kg|day		10/5# mg|kg|day	
Week	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 50	3	5.00	3	5.00	8	13.33	9	15.00
51 - 80	21	40.00	23	43.33	23	51.67	26	58.33
81 - 96	16	66.67	12	63.33	15	76.67	8	71.67
Ter. Sac.	20	33.33	22	36.67	14	23.33	17	28.33
Total	60	100.00	60	100.00	60	100.00	60	100.00

#: The dose level for Group 4 was decreased to 5 mg/kg/day beginning Week 13 (Day 86), and dose level for Group 3 was decreased to 2 mg/kg/day beginning Week 14 (Day 92).

Figure 1A: Kaplan-Meier Survival Curves for **Male Rats**

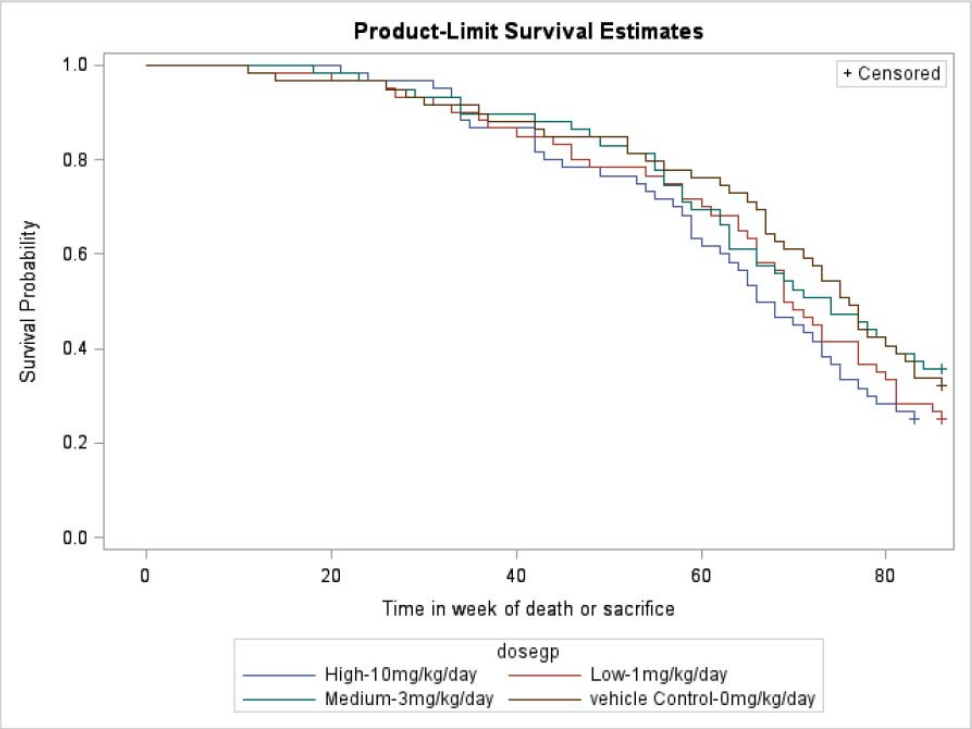
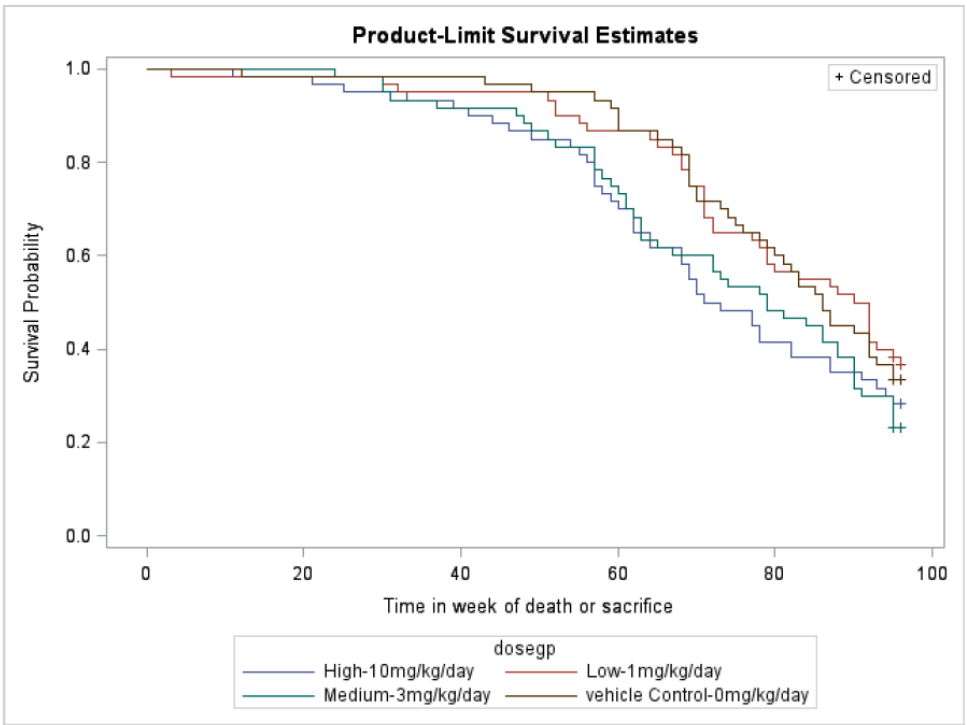


Figure 1B: Kaplan-Meier Survival Curves **for Female Rats**



Clinical signs: Test article-related clinical observations included soft and liquid feces (as expected) and brown fur (related to the changes in feces) in all treated groups and brown skin staining in Group 3 (3/2 mg/kg/day) and Group 4 (10/5 mg/kg/day) males.

Body weight: AZ1722-related decreases in body weight were noted in males and females at 10/5 mg/kg/day compared with controls beginning on Day 4 and continued for the duration of treatment, and the differences were generally statistically significant. The mean body weight of males in the 3/2 mg/kg/day dose group and females in the 1 and 3/2 mg/kg/day dose groups showed a statistically significant decrease in body weight on Days 67 and 22, respectively that continued throughout the study.

The mean body weights of male and female rats are shown in the Table below.

Table: Body weight of male and female rats (g)

	Days	Group 1 (0 mg/kg/day)	Group 2 (1 mg/kg/day)	Group 3 (3/2 mg/kg/day)	Group 4 (10/5 mg/kg/day)
Male	1	251.6±14.2	249±13.9	249.4±13.7	246.6±12.0
	4	277.8±15.4	277.1±15.8	273.9±14.0	267.8±12.6***
	57	585.5±50.8	580.0±48.9	568.6±45.1	512.9±45.5***
	67	611±54.9	598.5±54.5	561.7±48.4***	488.9±57.8***
	92	663.4±64	651.6±57.9	625.7±55.2**	600.0±51.2***
	204	808.2±100.8	791.6±84.6	764.2±84.7*	734.3±76.8***
	358	903.9±104.1	881.2±105.4	841.7±114.7**	811.7±91.4***
	470	902.7±104.4	884.6±137.8	884.7±124.8	819.1±112.7*
	554	891.8±118.9	909.8±125.2	868.7±140.4	828.4±113.5
Female	1	191.0±10.9	189.3±10.5	189.5±11.2	187.2±10.3
	4	203.8±12.6	202.3±12.1	202.8±12.1	197.9±12.0*
	57	320.0±26.1	295.3±28.5***	296.6±23.2***	289.8±23.7***
	67	328.1±29.3	297.6±27.4***	298.2±25.9***	260.4±33.3***
	92	346.1±33.3	316.9±30.3***	316.8±26.4	315.4±25.8***
	204	421.6±53.2	372.0±46.2***	373.3±37.6***	364.6±39.7***
	358	511.7±79.5	422.5±60.4***	436.4±55.9***	412.1±50.3***
	470	532.3±100.8	441.7±60.5***	456.5±56.3**	418.3±62.7***
	667	641.4±114.0	472.2±68.0**	574.7±61.2	435.9±102.6***

*=Significantly different from control ($p<0.05$)
** =Significantly different from control ($p<0.01$)
*** = Significantly different from control ($p<0.001$)

The following figures (from page 54 and 56 of the study report) show the growth curves for males and females.

Figure: Growth curve of Males.

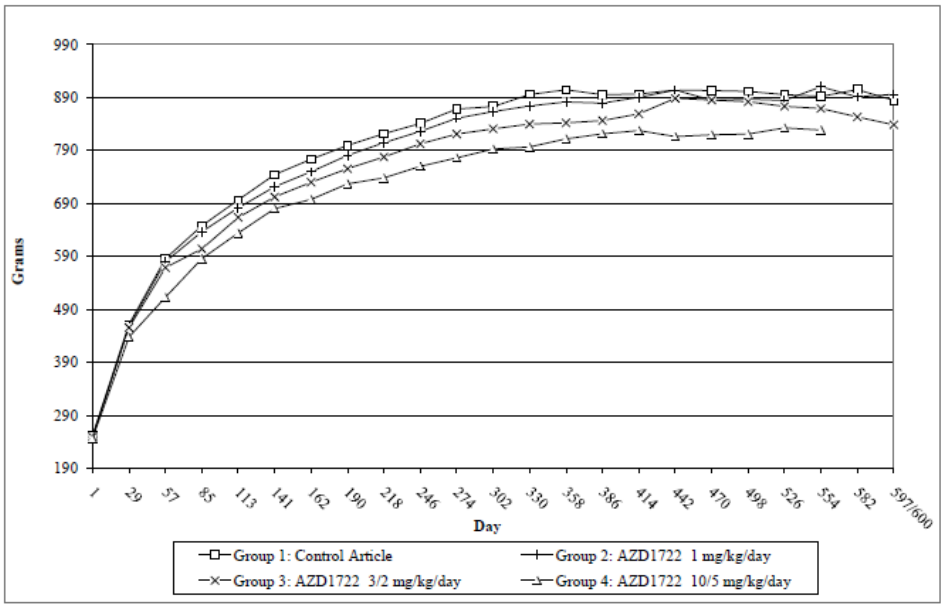
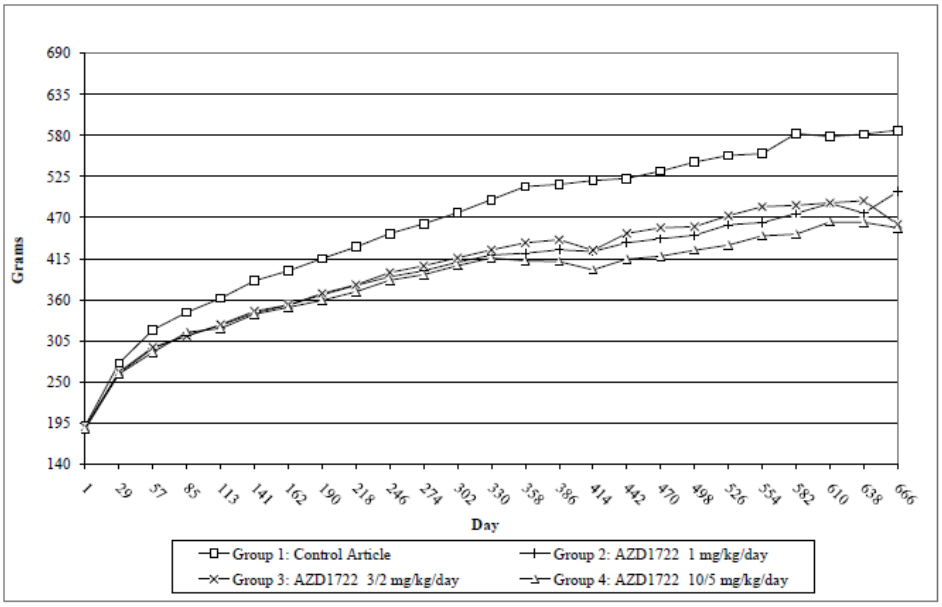


Figure: Growth curve of Females.



Food consumption: Mean food consumption of males and females at the 10/5 mg/kg/day dose was significantly lower prior to the dosing holiday beginning on Day 69 (22% vs control). During the dosing holiday for Group 4, food consumption was notably increased. After dosing resumed (Days 86 and 92), mean food consumption was still decreased (sometimes statistically) over the course of the study for Group 4 males and females. In addition, mean food consumption was generally lower for Group 2 and 3 females compared to control during the study.

The following figures (from page 58 and 60 of the study report) show the summary of food consumption for males and females.

Figure: Food consumption Males (g).

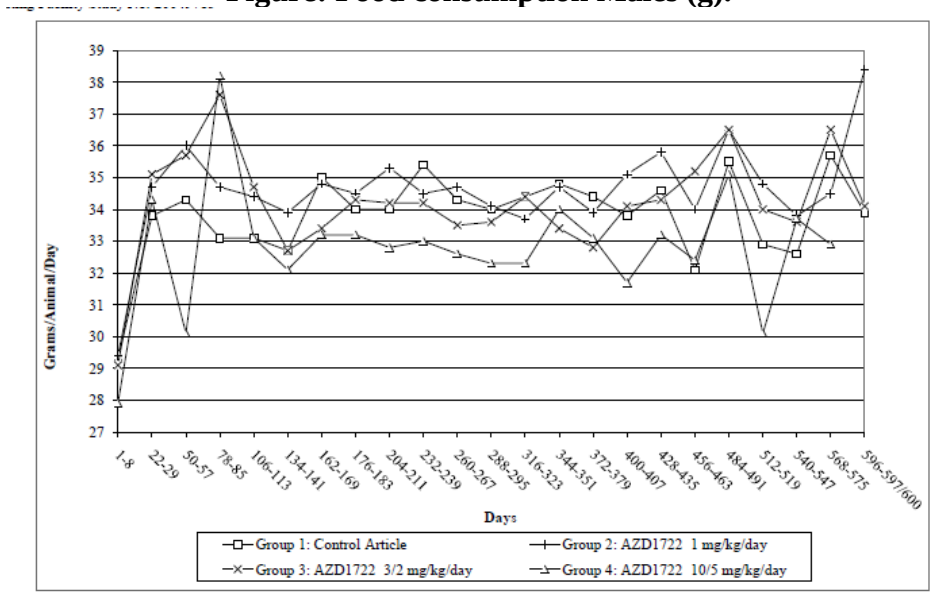
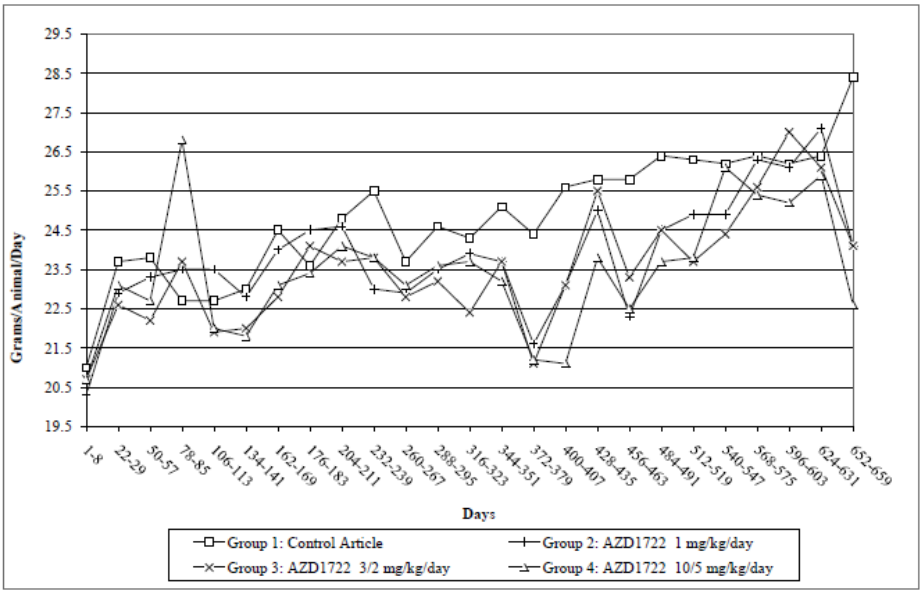


Figure: Food consumption Females (g).



Ophthalmoscopy: There were no test article-related ophthalmic changes noted.

Hematology: There were no test article-related changes in hematology parameters.

Serum Chemistry: Serum sodium concentration was measured in the toxicokinetic animals only prior to termination during Week 52. Although statistical analysis was limited due to the number of remaining animals, sodium values were similar among the groups.

Gross pathology: Test article-related gross pathology findings were noted in the gastrointestinal tract (stomach, duodenum, jejunum, ileum, cecum, colon, and rectum) and mesenteric lymph nodes. An increased incidence of abnormal content (dark, green, mucoid, watery, gritty, and/or soft material) was noted in the males and females at all dose levels. The increased incidence of abnormal content was generally non-dose-responsive, was higher in males than females, and exhibited the highest incidence in the cecum and colon compared to other portions of the gastrointestinal tract. Perforation of the cecum in females at 10/5 mg/kg/day was also correlated microscopically with perforation in 1 of the affected animals. Dilatation was noted in the stomach of males at ≥1 mg/kg/day; duodenum of males and females at ≥3/2 mg/kg/day; and jejunum, ileum, cecum, and colon of males and females at ≥1 mg/kg/day. Dark discoloration was noted in the ileum and cecum of males at ≥1 mg/kg/day and in the cecum of females at ≥1 mg/kg/day. Dark discoloration or dark focus was noted at a higher incidence in males compared to females and correlated microscopically with hemorrhage or mixed cell inflammation in these tissues.

Histopathology:

Non-neoplastic**:** Non-neoplastic microscopic findings related to AZD1722 administration were noted in the gastrointestinal tract (jejunum, ileum, cecum, and colon) and mesenteric lymph node. In the cecum, minimal to severe hemorrhage and mixed cell inflammation in males and females at ≥1 mg/kg/day with a dose-responsive increase in the incidence and/or severity were noted. Moderate perforation in 1 female at 10/5 mg/kg/day, and ulceration in 2 males at 3/2 mg/kg/day and 1 female at 10/5 mg/kg/day dose groups were observed. In the ileum, minimal to marked hemorrhage and mixed cell inflammation in males at ≥1 mg/kg/day, minimal hemorrhage in 1 female at 10/5 mg/kg/day, and mild mixed cell inflammation in 1 female at 1 mg/kg/day dose group were noted. In the colon, AZD1722 caused non-dose-responsive hemorrhage in males at ≥3/2 mg/kg/day (mild to moderate) and 1 female at 1 mg/kg/day (minimal), an increased incidence of non-dose-responsive mixed cell inflammation in males at ≥3/2 mg/kg/day, and mild thrombosis in 1 male and 1 female each at 10/5 mg/kg/day. In the jejunum, minimal to mild hemorrhage in males at ≥1 mg/kg/day and mild mixed cell inflammation in 1 male at 3/2 mg/kg/day were noted. In the mesenteric lymph node, there was an increased incidence of erythrocytosis in the males and females at ≥1 mg/kg/day which correlated with dark discoloration of the lymph node at necropsy. Summary of non-neoplastic findings are presented in the Table below (from page 43 and 44 of the study report).

Summary of Noteworthy Non-Neoplastic Microscopic Findings – All Fates Combined

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)	**0**	**1**	**3/2**	**10/5**	**0**	**1**	**3/2**	**10/5**
No. animals examined	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
Jejunum (No. examined)	**46**	**44**	**43**	**39**	**53**	**51**	**50**	**44**
Hemorrhage	(0)	(1)	(1)	(1)	(0)	(0)	(0)	(0)
Minimal	-	1	0	0	-	-	-	-
Mild	-	0	1	1	-	-	-	-
Inflammation, mixed cell	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Minimal	-	-	0	-	-	-	-	-
Mild	-	-	1	-	-	-	-	-
Ileum (No. examined)	**47**	**47**	**45**	**44**	**53**	**56**	**51**	**42**
Hemorrhage	(0)	(4)	(3)	(5)	(0)	(0)	(0)	(1)
Minimal	-	2	0	2	-	-	-	1
Mild	-	2	2	2	-	-	-	0
Moderate	-	0	1	0	-	-	-	0
Marked	-	0	0	1	-	-	-	0
Inflammation, mixed cell	(0)	(2)	(6)	(3)	(0)	(1)	(0)	(0)
Minimal	-	0	1	1	-	0	-	-
Mild	-	2	3	2	-	1	-	-
Moderate	-	0	0	0	-	0	-	-
Marked	-	0	2	0	-	0	-	-
Cecum (No. examined)	**45**	**46**	**51**	**50**	**54**	**53**	**51**	**53**
Hemorrhage	(0)	(13)	(16)	(19)	(0)	(4)	(10)	(17)
Minimal	-	5	2	8	-	1	3	4
Mild	-	2	5	6	-	3	3	6
Moderate	-	3	2	2	-	0	2	6
Marked	-	3	6	2	-	0	1	0
Severe	-	0	1	1	-	0	1	1
Inflammation, mixed cell	(0)	(11)	(18)	(17)	(1)	(4)	(8)	(18)
Minimal	-	5	5	4	1	3	3	10
Mild	-	2	7	9	0	1	4	3
Moderate	-	3	2	4	0	0	0	4
Marked	-	1	3	0	0	0	1	1
Severe	-	0	1	0	0	0	0	0
Necrosis	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Minimal	-	-	-	0	-	-	-	-
Mild	-	-	-	1	-	-	-	-

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)	**0**	**1**	**3/2**	**10/5**	**0**	**1**	**3/2**	**10/5**
No. animals examined	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
Perforation	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
Minimal	-	-	-	-	-	-	-	0
Mild	-	-	-	-	-	-	-	0
Moderate	-	-	-	-	-	-	-	1
Ulceration	(0)	(0)	(2)	(0)	(0)	(0)	(0)	(1)
Minimal	-	-	0	-	-	-	-	1
Mild	-	-	0	-	-	-	-	0
Moderate	-	-	0	-	-	-	-	0
Marked	-	-	2	-	-	-	-	0
Colon (No. examined)	**60**	**51**	**51**	**52**	**60**	**58**	**57**	**56**
Hemorrhage	(0)	(0)	(2)	(1)	(0)	(1)	(0)	(0)
Minimal	-	-	0	0	-	1	-	-
Mild	-	-	1	0	-	0	-	-
Moderate	-	-	1	1	-	0	-	-
Inflammation, mixed cell	(1)	(1)	(5)	(4)	(0)	(0)	(0)	(0)
Minimal	1	1	2	2	-	-	-	-
Mild	0	0	1	1	-	-	-	-
Moderate	0	0	2	1	-	-	-	-
Thrombosis	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(1)
Minimal	-	-	-	0	-	-	-	0
Mild	-	-	-	1	-	-	-	1
Lymph node, mesenteric (No. examined)	**60**	**60**	**60**	**59**	**59**	**60**	**60**	**60**
Erythrocytosis, medullary sinus	(3)	(10)	(11)	(13)	(2)	(3)	(7)	(10)
Minimal	3	5	3	7	2	2	3	5
Mild	0	3	4	4	0	0	4	5
Moderate	0	1	2	1	0	1	0	0
Marked	0	1	2	1	0	0	0	0

Note: "-" indicates the severity is not applicable due to absence of the finding in the group.

Neoplastic**:** There were no neoplastic changes related to AZD1722 administration indicating no test article-related carcinogenic effects in rats. However, incidence of endometrial stromal sarcoma in the cervix at 1 mg/kg/day (3/60; 5.0%, not statistically significant) was higher than the control group (0/60) and higher than the historical range for this neoplasm at this Testing Facility (up to 1.7%). There was no dose-responsive trend (1/60 and 0/60 at 3/2 mg/kg/day and 10/5 mg/kg/day, respectively) for the endometrial stromal sarcoma incidences. Similarly, the number of benign mixed sex cord stromal tumors in the ovary, a tumor which is not listed in the historical control data at this Testing Facility, was higher in the 1 mg/kg/day (3/60; 5.0%) and 10/5 mg/kg/day (2/60; 3.3%) groups than in the concurrent control group (0/60). This tumor incidences in the ovary was also not statistically significant and lacked a dose-responsive trend and was therefore considered unrelated to AZD1722 administration. The tumor incidence summary is presented in the Table below (page 16-21 from statistical review by Malick Mbodj, Ph.D.).

Table3A: Tumor Rates and P-Values for Dose Response Relationship and the pairwise comparisons

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Veh. Cont (N=60) P - Trend	1 mg Low (N=60) P - VC vs. L	3/2[#] mg Med (N=60) P -VC vs. M	10/5[#] mg High (N=60) P - VC vs. H
Brain	Astrocytoma, Malignant	0/59 (21) 0.2208	0/60 (19) NC	0/59 (20) NC	1/60 (17) 0.4474
	Meningioma, Malignant	1/59 (21) 0.3951	0/60 (19) 1.0000	0/59 (20) 1.0000	1/60 (17) 0.7013
Gland, Adrenal	Cortical Adenoma	1/59 (21) 0.7403	0/60 (19) 1.0000	1/59 (21) 0.7561	0/60 (17) 1.0000
	Pheochromocytoma, Benign	3/59 (22) 0.8151	2/60 (20) 0.7971	1/59 (20) 0.9346	1/60 (17) 0.9111
	Pheochromocytoma, Malignant	1/59 (21) 0.7334	0/60 (19) 1.0000	1/59 (20) 0.7439	0/60 (17) 1.0000
	Pheochromocytoma Benign/Malignant	4/59 (23) 0.8587	2/60 (20) 0.8731	2/59 (21) 0.8856	1/60 (17) 0.9489
Gland, Mammary	Adenocarcinoma	0/52 (18) 0.7391	1/53 (18) 0.5000	0/52 (18) NC	0/54 (15) NC
	Adenoma	0/52 (18) 0.7391	1/53 (18) 0.5000	0/52 (18) NC	0/54 (15) NC
	Adenoma/Adenocarcinoma	0/59 (21) 0.7902	2/60 (20) 0.2317	0/59 (20) NC	0/60 (17) NC
	Fibroadenoma	5/52 (21) 0.9894	3/53 (20) 0.8656	2/52 (19) 0.9385	0/54 (15) 1.0000
Gland, Parathyroid	Adenoma	0/51 (18) 0.5000	0/45 (14) NC	1/51 (18) 0.5000	0/51 (14) NC
Gland, Pituitary	Adenoma	27/58 (38) 0.2943	21/60 (31) 0.7129	31/59 (39) 0.2765	24/60 (32) 0.4616
	Carcinoma	2/58 (21) 0.6842	1/60 (19) 0.8654	0/59 (20) 1.0000	1/60 (17) 0.8423
	Adenoma/Carcinoma	29/59 (39) 0.3646	22/60 (32) 0.7848	31/59 (39) 0.3944	25/60 (33) 0.5557
Gland, Prostate	Adenocarcinoma	0/59 (21) 0.2208	0/60 (19) NC	0/59 (20) NC	1/60 (17) 0.4474
Gland, Thyroid	C-Cell Adenoma	5/59 (23) 0.5165	4/60 (21) 0.7223	3/59 (21) 0.8486	4/60 (19) 0.6639
	C-Cell Carcinoma	3/59 (23) 0.8386	2/60 (20) 0.7811	0/59 (20) 1.0000	1/60 (17) 0.9031
	Adenoma/Carcinoma C-Cell	8/59 (25) 0.6475	5/60 (21) 0.8268	3/59 (21) 0.9624	5/60 (19) 0.7698
	Follicular Cell Adenoma	3/59 (22) 0.9605	3/60 (20) 0.6204	2/59 (21) 0.8131	0/60 (17) 1.0000
Heart	Mesothelioma, Malignant	1/59 (21) 1.0000	0/60 (19) 1.0000	0/59 (20) 1.0000	0/60 (17) 1.0000
Hemolymphoreticular Tissue	Histiocytic Sarcoma	1/59 (21) 0.9282	1/60 (19) 0.7308	0/59 (20) 1.0000	0/60 (17) 1.0000
	Leukemia, Granulocytic	0/59 (21) 0.2208	0/60 (19) NC	0/59 (20) NC	1/60 (17) 0.4474
	Lymphoma, Malignant	1/59 (22) 0.7339	0/60 (19) 1.0000	1/59 (21) 0.7442	0/60 (17) 1.0000

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Veh. Cont (N=60) P - Trend	1 mg Low (N=60) P - VC vs. L	3/2[a] mg Med (N=60) P -VC vs. M	10/5[a] mg High (N=60) P - VC vs. H
Kidney	Adenoma	1/59 (21) 0.1314	0/60 (19) 1.0000	0/59 (20) 1.0000	2/60 (18) 0.4409
	Tubular Cell Adenoma	1/59 (21) 1.0000	0/60 (19) 1.0000	0/59 (20) 1.0000	0/60 (17) 1.0000
Liver	Cholangiocarcinoma	0/59 (21) 0.2208	0/60 (19) NC	0/59 (20) NC	1/60 (17) 0.4474
	Hepatocellular Adenoma	1/59 (21) 0.9282	1/60 (19) 0.7308	0/59 (20) 1.0000	0/60 (17) 1.0000
	Hepatocellular Carcinoma	0/59 (21) 0.2533	1/60 (19) 0.4750	1/59 (20) 0.4878	1/60 (17) 0.4474
	Hepatocellular Adenoma/Carcinoma	1/59 (21) 0.5529	2/60 (20) 0.4812	1/59 (20) 0.7439	1/60 (17) 0.7013
Lymph Node, Mesenteric	Hemangiosarcoma	0/59 (21) 0.5934	1/60 (19) 0.4750	1/59 (21) 0.5000	0/59 (17) NC
Pancreas	Adenoma	0/59 (21) 0.1642	0/60 (19) NC	1/59 (21) 0.5000	1/60 (17) 0.4474
	Carcinoma	0/59 (21) 0.4805	0/60 (19) NC	1/59 (20) 0.4878	0/60 (17) NC
	Adenoma/Carcinoma	0/59 (21) 0.1649	0/60 (19) NC	2/59 (21) 0.2439	1/60 (17) 0.4474
	Islet Cell Adenoma	5/59 (23) 0.7694	4/60 (21) 0.7223	7/59 (23) 0.3691	2/60 (18) 0.9083
	Islet Cell Carcinoma	1/59 (21) 0.7334	0/60 (19) 1.0000	1/59 (20) 0.7439	0/60 (17) 1.0000
	Islet Cell Adenoma/Carcinoma	6/59 (23) 0.8287	4/60 (21) 0.8197	8/59 (24) 0.4120	2/60 (18) 0.9487
Skin	Fibroma	2/59 (21) 1.0000	0/60 (19) 1.0000	0/59 (20) 1.0000	0/60 (17) 1.0000
	Fibrosarcoma	0/59 (21) 0.7308	1/60 (20) 0.4878	0/59 (20) NC	0/60 (17) NC
	Keratoacanthoma	2/59 (21) 0.7605	3/60 (21) 0.5000	1/59 (20) 0.8752	1/60 (17) 0.8423
	Leiomyosarcoma	0/59 (21) 0.4805	0/60 (19) NC	1/59 (20) 0.4878	0/60 (17) NC
	Osteosarcoma	0/59 (21) 0.2308	0/60 (19) NC	0/59 (20) NC	1/60 (18) 0.4615
	Papilloma	1/59 (21) 0.9273	2/60 (20) 0.4812	0/59 (20) 1.0000	0/60 (17) 1.0000
	Schwannoma, Malignant	0/59 (21) 0.7308	1/60 (20) 0.4878	0/59 (20) NC	0/60 (17) NC
	Squamous Cell Carcinoma	3/59 (22) 1.0000	0/60 (19) 1.0000	0/59 (20) 1.0000	0/60 (17) 1.0000
	Squamous Cell Carcinoma/Keratoacanthoma/	6/59 (23) 0.9821	5/60 (22) 0.7277	1/59 (20) 0.9924	1/60 (17) 0.9869
Spleen	Hemangiosarcoma	0/59 (21) 0.7308	1/60 (20) 0.4878	0/59 (20) NC	0/60 (17) NC
Stomach	Adenocarcinoma	0/59 (21) 0.2208	0/59 (19) NC	0/59 (20) NC	1/58 (17) 0.4474

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Veh. Cont (N=60) P - Trend	1 mg Low (N=60) P - VC vs. L	3/2# mg Med (N=60) P -VC vs. M	10/5# mg High (N=60) P - VC vs. H
	Papilloma	0/59 (21) 0.5934	1/59 (19) 0.4750	1/59 (21) 0.5000	0/58 (17) NC
Tail	Keratoacanthoma	1/6 (3) 0.9333	1/5 (3) 0.8000	0/8 (3) 1.0000	0/3 (1) 1.0000
	Leiomyosarcoma	0/6 (3) 0.5000	0/5 (2) NC	1/8 (4) 0.5714	0/3 (1) NC
Urinary Bladder	Transitional Cell Carcinoma	0/59 (21) 0.4737	0/60 (19) NC	1/59 (20) 0.4878	0/59 (16) NC

& 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

Table 3B: Tumor Rates and P-Values for Dose Response Relationship and the pairwise comparisons

Female Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Veh. Cont. (N=60) P - Trend	1 mg Low (N=60) P -VC vs. L	3/2# mg Med (N=60) P -VC vs. M	10/5# mg High (N=60) P -VC vs. H
Bone, Sternum	Osteoblastoma, Benign	0/60 (31) 0.2212	0/60 (31) NC	0/60 (26) NC	1/60 (25) 0.4464
Brain	Astrocytoma, Malignant	0/60 (31) 0.2212	0/60 (31) NC	0/60 (26) NC	1/60 (25) 0.4464
Cervix	Endometrial Stromal Sarcoma	0/60 (31) 0.7519	3/60 (33) 0.1310	1/60 (27) 0.4655	0/60 (25) NC
	Granular Cell Tumor, Benign	0/60 (31) 0.7738	2/60 (32) 0.2540	0/60 (26) NC	0/60 (25) NC
	Schwannoma, Malignant	1/60 (32) 0.9230	1/60 (31) 0.7460	0/60 (26) 1.0000	0/60 (25) 1.0000
Gland, Adrenal	Cortical Adenoma	3/60 (32) 0.6835	0/60 (31) 1.0000	0/60 (26) 1.0000	1/60 (25) 0.9090
	Cortical Carcinoma	0/60 (31) 0.7257	1/60 (31) 0.5000	0/60 (26) NC	0/60 (25) NC
	Cortical Adenoma/Carcinoma	3/60 (32) 0.7538	1/60 (31) 0.9396	0/60 (26) 1.0000	1/60 (25) 0.9090
	Pheochromocytoma, Benign	0/60 (31) 0.0819	1/60 (32) 0.5079	0/60 (26) NC	2/60 (25) 0.1948
	Pheochromocytoma, Malignant	1/60 (31) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
	Pheochromocytoma Benign/Malignant	1/60 (31) 0.1877	1/60 (32) 0.7619	0/60 (26) 1.0000	2/60 (25) 0.4185
Gland, Mammary	Adenocarcinoma	20/60 (41) 0.7144	18/60 (40) 0.7133	15/60 (33) 0.6978	12/60 (29) 0.8037
	Adenoma	11/60 (38) 0.8875	7/60 (33) 0.8463	5/60 (28) 0.9097	4/60 (26) 0.9432
	Adenoma/Adenocarcinoma	26/60 (45) 0.8725	23/60 (41) 0.6463	19/60 (35) 0.7054	13/60 (29) 0.9080
	Carcinosarcoma	1/60 (32) 0.9243	1/60 (32) 0.7540	0/60 (26) 1.0000	0/60 (25) 1.0000
	Fibroadenoma	17/60 (39) 0.2934	15/60 (38) 0.7248	9/60 (29) 0.9048	15/60 (31) 0.4367
Gland, Parathyroid	Adenoma	1/46 (25) 0.7981	1/50 (25) 0.7551	1/50 (22) 0.7225	0/54 (21) 1.0000
Gland, Pituitary	Adenoma	49/60 (56) 0.9625	46/60 (52) 0.5575	40/59 (46) 0.6498	31/60 (41) 0.9629
	Carcinoma	2/60 (32) 0.5985	1/60 (32) 0.8810	1/59 (26) 0.8393	1/60 (25) 0.8305
	Adenoma/Adenocarcinoma	51/60 (56) 0.9705	47/60 (53) 0.7676	41/60 (47) 0.8284	32/60 (41) 0.9817
Gland, Thyroid	C-Cell Adenoma	7/60 (34) 0.8865	8/60 (34) 0.5000	2/60 (27) 0.9687	3/60 (26) 0.9019
	C-Cell Carcinoma	4/60 (33) 0.9985	1/60 (31) 0.9689	0/60 (26) 1.0000	0/60 (25) 1.0000
	Adenoma/Carcinoma C-Cell	11/60 (36) 0.9785	9/60 (35) 0.7630	2/60 (27) 0.9966	3/60 (26) 0.9838

Female Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Veh. Cont. (N=60) P - Trend	1 mg Low (N=60) P -VC vs. L	3/2# mg Med (N=60) P -VC vs. M	10/5# mg High (N=60) P -VC vs. H
	Follicular Cell Adenoma	0/60 (31) 0.1501	0/60 (31) NC	1/60 (26) 0.4561	1/60 (25) 0.4464
	Follicular Cell Carcinoma	0/60 (31) 0.4561	0/60 (31) NC	1/60 (27) 0.4655	0/60 (25) NC
	Adenoma/Carcinoma Follicular Cell	0/60 (31) 0.1571	0/60 (31) NC	2/60 (27) 0.2123	1/60 (25) 0.4464
Heart	Hemangiosarcoma	0/60 (31) 0.2212	0/60 (31) NC	0/60 (26) NC	1/60 (25) 0.4464
Hemolymphoreticular Tissue	Histiocytic Sarcoma	1/60 (31) 0.7064	0/60 (31) 1.0000	1/60 (27) 0.7187	0/60 (25) 1.0000
	Leukemia, Large Granular Lymphocytic	0/60 (31) 0.4561	0/60 (31) NC	1/60 (27) 0.4655	0/60 (25) NC
	Lymphoma, Malignant	0/60 (31) 0.6773	2/60 (32) 0.2540	1/60 (26) 0.4561	0/60 (25) NC
Kidney	Adenoma	2/60 (32) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
	Tubular Cell Adenoma	1/60 (32) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
	Tubular Cell Carcinoma	1/60 (31) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
	Tubular Cell Adenoma/Carcinoma	2/60 (32) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
Liver	Cholangiocarcinoma	0/60 (31) 0.4513	0/60 (31) NC	1/60 (26) 0.4561	0/60 (25) NC
	Hemangiosarcoma	1/60 (31) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
	Hepatocellular Adenoma	0/60 (31) 0.6773	2/60 (32) 0.2540	1/60 (26) 0.4561	0/60 (25) NC
Lung	Bronchioloalveolar Carcinoma	0/60 (31) 0.7257	1/60 (31) 0.5000	0/60 (26) NC	0/60 (25) NC
	Squamous Cell Carcinoma	0/60 (31) 0.4561	0/60 (31) NC	1/60 (27) 0.4655	0/60 (25) NC
Ovary	Mesothelioma, Malignant	0/60 (31) 0.2281	0/60 (31) NC	0/60 (26) NC	1/60 (26) 0.4561
	Mixed Sex Cord Stromal Tumor, Benign	0/60 (31) 0.2057	3/60 (32) 0.1249	0/60 (26) NC	2/60 (25) 0.1948
	Thecoma, Malignant	1/60 (31) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
Pancreas	Adenoma	0/60 (31) 0.4513	0/60 (31) NC	1/60 (26) 0.4561	0/60 (25) NC
	Islet Cell Adenoma	3/60 (32) 0.4637	1/59 (31) 0.9396	0/60 (26) 1.0000	2/60 (26) 0.7520
	Islet Cell Carcinoma	1/60 (31) 0.9192	2/59 (31) 0.5000	0/60 (26) 1.0000	0/60 (25) 1.0000
	Islet Cell Adenoma/Carcinoma	4/60 (33) 0.7127	3/59 (32) 0.7739	0/60 (26) 1.0000	2/60 (26) 0.8385
Skin	Basal Cell Tumor, Benign	1/60 (31) 0.3951	0/60 (31) 1.0000	0/60 (26) 1.0000	1/60 (25) 0.6981

Female Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Veh. Cont. (N=60) P - Trend	1 mg Low (N=60) P -VC vs. L	3/2# mg Med (N=60) P -VC vs. M	10/5# mg High (N=60) P -VC vs. H
	Fibrosarcoma	0/60 (31) 0.4513	0/60 (31) NC	1/60 (26) 0.4561	0/60 (25) NC
	Papilloma	0/60 (31) 0.0505	0/60 (31) NC	0/60 (26) NC	2/60 (26) 0.2036
	Schwannoma, Malignant	0/60 (31) 0.2212	0/60 (31) NC	0/60 (26) NC	1/60 (25) 0.4464
Spleen	Hemangiosarcoma	0/60 (31) 0.7257	1/60 (31) 0.5000	0/60 (26) NC	0/60 (25) NC
Stomach	Leiomyosarcoma	0/60 (31) 0.4464	0/58 (31) NC	1/60 (27) 0.4655	0/56 (23) NC
	Squamous Cell Carcinoma	1/60 (31) 1.0000	0/58 (31) 1.0000	0/60 (26) 1.0000	0/56 (23) 1.0000
Uterus	Endometrial Stromal Polyp	1/60 (31) 0.4073	4/60 (33) 0.1975	0/60 (26) 1.0000	2/60 (25) 0.4185
	Granular Cell Tumor, Benign	1/60 (31) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
	Leiomyosarcoma	1/60 (32) 1.0000	0/60 (31) 1.0000	0/60 (26) 1.0000	0/60 (25) 1.0000
Vagina	Granular Cell Tumor, Benign	7/59 (33) 0.9029	4/59 (32) 0.8982	3/60 (27) 0.9203	2/60 (25) 0.9638

& 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

Toxicokinetics: Due to an insufficient number of quantifiable plasma sample concentrations, the toxicokinetic evaluation and the incurred sample re-analysis were not conducted.

Serology: All serological results were negative.

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The methodology and the conduct of the study appear to be appropriate and acceptable. The high-dose was selected on the basis of the MTD from the 14-day and 6-month toxicology studies in rats, and was concurred with by the Executive CAC. The test model (Crl:CD®(SD) rats) selection was in concurrence with the Agency. Overall, the study is valid and acceptable.

Evaluation of tumor findings: There was no tenapanor-related increase in any tumor incidences in male and female rats.

Appendix:

APPENDIX-1: ExecCAC Meeting Minutes dated November 17, 2016.
APPENDIX-2: Neoplastic findings in male and female mice
APPENDIX-3: ExecCAC Meeting Minutes dated December 17, 2013.
APPENDIX-4: ExecCAC Meeting Minutes dated January 22, 2019.

APPENDIX-1: ExecCAC Meeting Minutes dated November 17, 2016 (Mice)

Executive CAC

Date of Meeting: November 15, 2016

Committee: Karen Davis Bruno, Ph.D., OND-IO, Chair
Abby Jacobs, Ph.D., OND-IO, Member
Tim McGovern, Ph.D., OND-IO, Member
Lee Elmore, Ph.D., DMEP, Alternate Member
Owen McMaster, Ph.D., DAIP, Member
Sushanta K. Chakder, Ph.D., DGIEP, Supervisor
Dinesh Gautam, Ph.D., DGIEP, Presenting Reviewer

Author of Draft: Dinesh Gautam, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The Committee did not address the sponsor's proposed statistical evaluation for the carcinogenicity bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Standardized Study Data (December 2014) and the latest Study Data Technical Conformance Guide.

IND: 108,732
Drug Name: Tenapanor (AZD1722/RDX5791)
Sponsor: Ardelyx, Inc., Fremont, CA, USA

Background:
AZD1722, a sodium/hydrogen antiporter (NHE3) inhibitor, is being developed for the treatment of (b) (4) constipation-predominant irritable bowel syndrome (IBS-C), (b) (4) The Sponsor has submitted the Special Protocol Assessment (SPA) for a 6-month oral carcinogenicity study in male and female Tg.rasH2 mice.

RDX5791 was not genotoxic in the Ames bacterial reverse mutation assay, the *in vitro* human lymphocyte chromosomal aberration assay and the mouse micronucleus assay. AZ13792925 (the major metabolite of tenapanor) was non genotoxic in the Ames bacterial reverse mutation assay and the *in vitro* L5178Y $TK^{+/-}$ mouse lymphoma assay.

Tg.RasH2 Mouse Carcinogenicity Study Dose Selection

(b) (4)

Executive CAC Recommendations and Conclusions:

1. The Committee did not concur with the Sponsor's proposed doses.

2. For tenapanor, the Committee recommended doses of 0 (vehicle), 100, 300 and 800 mg/kg/day (0, 50, 150 and 400 mg/kg BID) by oral gavage for female Tg.RasH2 mice, with the high dose based on the MFD.

   For tenapanor, the Committee recommended doses of 0 (vehicle), 10, 30 and 100 mg/kg/day (0, 5, 15 and 50 mg/kg BID), by oral gavage for male Tg.RasH2 mice, with the high dose based on body weight loss in the mid and high dose groups in the 1-month dose range finding study.

3. For the metabolite, AZ13792915 the Committee recommended doses of 0 (vehicle), 55 and 165 mg/kg/day, by oral gavage for both male and female mice, with the high dose based on deaths at 500 mg/kg/day in the 1-month dose-ranging study.

4. The Committee noted that proposed vehicle for the carcinogenicity study is different from that used in the dose range finding studies for tenapanor and AZ13792915, respectively. The above recommendations by the Committee are based on using the same vehicles in the 6-month carcinogenicity study as in the dose range finding studies for tenapanor and AZ13792915, respectively. If the Sponsor decides to use their proposed vehicle in the 6-month carcinogenicity study, they need to conduct a new dose-ranging study using this vehicle.

5. The Committee recommended inclusion of a water or saline control group in addition to the vehicle control group/s.

Karen Davis Bruno, Ph.D.
Chair, Executive CAC

cc:\

/Division File, DGIEP
/DGautam, DGIEP
/SChakder, DGIEP
/RPM/CCherry-France, DGIEP
/ASeifried, OND-IO

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

KAREN L DAVIS BRUNO
11/17/2016

Reference ID: 4015417

Appendix 2: Neoplastic findings in male and female mice.

Provantis 8 - Production US

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- MALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
ARTERY, AORTA;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	9
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	4
Mesothelioma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	0
ARTERY, MESENTERIC;									
Examined	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Thrombus	0	1	0	0	0	0	0	1	0
BODY CAVITY, NASAL;									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
BODY CAVITY, ORAL;									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Squamous cell carcinoma; malignant without metastasis; fatal	0	0	0	0	0	0	1	0	0
BONE MARROW;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	11
Increased myeloid to erythroid ratio	0	0	0	0	0	0	0	0	0
Decreased cellularity	0	0	0	0	0	0	0	0	1
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
BONE, FEMUR;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	13
BONE, STERNUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)

APPEARS THIS WAY ON ORIG NAL

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- MALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
BONE, STERNUM; (continued)									
Within Normal Limits	25	25	25	25	25	25	25	25	13
BRAIN;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	8
Lymphoma, malignant; malignant; secondary;									
incidental	0	0	0	0	0	0	0	0	5
CERVIX;									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-
Hemangiosarcoma; malignant without metastasis;									
fatal	-	-	-	-	-	-	-	-	-
Schwannoma, malignant; malignant; secondary;									
incidental	-	-	-	-	-	-	-	-	-
Lymphoma, malignant; malignant; secondary;									
incidental	-	-	-	-	-	-	-	-	-
EAR;									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Inflammation, neutrophilic; tympanic cavity;									
bilateral	0	0	0	0	0	0	0	0	0
EPIDIDYMIS;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	22	24	22	22	23	24	24	11
Sperm granuloma; unilateral	0	3	1	3	2	2	0	1	0
Decreased cellularity; lumen; sperm; bilateral	0	0	0	0	1	0	0	0	0
Dilatation; ductular; focal	0	0	0	0	0	0	1	0	0
Lymphoma, malignant; malignant; secondary;									
incidental	0	0	0	0	0	0	0	0	2
ESOPHAGUS;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	12

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- MALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
ESOPHAGUS; (continued)									
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	0
Squamous cell carcinoma; malignant without metastasis; fatal	0	0	0	0	0	0	0	0	1
EYE;									
Examined.....	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits.....	25	25	25	25	25	25	25	25	0
Degeneration; retina; bilateral	0	0	0	0	0	0	0	0	13
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
GALLBLADDER:									
Examined.....	(25)	(24)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits.....	25	24	25	25	25	25	25	25	13
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	1	0	0	0	0	0	0	0
GALT;									
Examined.....	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(24)	(13)
Within Normal Limits.....	25	25	25	25	25	25	25	24	13
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	1	0
GLAND, ADRENAL;									
Examined.....	(24)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits.....	13	18	13	15	13	17	19	18	9
Not Examined: NOT PRESENT IN WET TISSUES.	1	0	0	0	0	0	0	0	0
Hyperplasia; subcapsular cell; bilateral	6	2	3	2	3	2	0	0	2
Hyperplasia; subcapsular cell; unilateral	5	5	9	8	9	6	6	7	1
Congestion; bilateral	0	0	0	0	0	0	0	0	1
GLAND, HARDERIAN;									
Examined.....	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits.....	22	23	25	22	23	24	25	25	12
Hyperplasia; unilateral; focal	0	0	0	0	0	0	0	0	0
Infiltration, mononuclear cell	3	2	0	3	2	1	0	0	0
Adenoma; benign; incidental	0	0	0	0	0	0	0	0	0

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	MALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
GLAND, HARDERIAN; (continued)									
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
GLAND, LACRIMAL;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	22	24	22	22	25	22	25	24	13
Infiltration, mononuclear cell	3	1	3	3	0	3	0	1	0
GLAND, MAMMARY;									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
GLAND, PARATHYROID;									
Examined	(16)	(13)	(11)	(15)	(16)	(18)	(14)	(20)	(5)
Within Normal Limits	16	12	11	14	15	18	13	20	5
Not Examined: NOT PRESENT IN SECTION.	9	12	13	10	9	7	11	5	8
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	1	0	0	0	0	0	0
Cyst; unilateral	0	1	0	1	1	0	1	0	0
GLAND, PITUITARY;									
Examined	(24)	(25)	(24)	(25)	(25)	(25)	(25)	(24)	(12)
Within Normal Limits	24	25	24	25	25	25	25	24	12
Not Examined: EXHAUSTED DURING HISTOLOGY PROCESSING.	1	0	0	0	0	0	0	0	0
Not Examined: LOST DURING NECROPSY.	0	0	0	0	0	0	0	0	0
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	1
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	1	0	0	0	0	1	0
GLAND, PROSTATE;									
Examined	(25)	(22)	(25)	(24)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	22	25	24	25	25	25	25	11
Not Examined: NOT PRESENT IN SECTION.	0	3	0	1	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	2

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- MALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
GLAND, SALIVARY;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	23	23	22	21	21	22	18	12
Infiltration, mononuclear cell	0	2	2	3	4	4	3	7	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
GLAND, SEMINAL VESICLE;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	12
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
GLAND, THYROID;									
Examined	(25)	(24)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	24	25	25	25	25	25	25	12
Not Examined: NOT PRESENT IN SECTION.	0	1	0	0	0	0	0	0	1
GLAND, ZYMBALS;									
Examined	(25)	(25)	(23)	(17)	(20)	(24)	(23)	(21)	(10)
Within Normal Limits	25	25	23	17	20	24	23	21	10
Not Examined: NOT PRESENT IN SECTION.	0	0	2	8	5	1	2	4	3
HEART;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	24	25	25	25	25	7
Cardiomyopathy	0	0	0	1	0	0	0	0	1
Mesothelioma, malignant; malignant with metastasis ; primary; fatal	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	6
HEMOLYMPHORETICULAR TISSUE;									
Examined	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(10)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant with metastasis; primary; incidental	0	1	0	0	0	0	0	0	0

APPEARS THIS WAY ON ORIGINAL

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- MALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
HEMOLYMPHORETICULAR TISSUE; (continued)									
Lymphoma, malignant; malignant with metastasis; primary; fatal	0	0	0	0	0	0	0	0	10
KIDNEY;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	16	16	16	18	18	15	16	13	6
Infiltration, mononuclear cell	1	7	4	3	3	8	7	3	0
Infiltration, mononuclear cell; pelvis	7	1	5	4	3	2	2	9	0
Degeneration/regeneration; tubular	2	1	0	0	1	0	0	0	0
Inflammation, mixed cell; multifocal	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	7
LARGE INTESTINE, CECUM;									
Examined	(25)	(25)	(25)	(24)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	24	25	25	25	25	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	1	0	0	0	0	0
Single cell necrosis; mucosal	0	0	0	0	0	0	0	0	1
LARGE INTESTINE, COLON;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	13
LARGE INTESTINE, RECTUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	13
LARYNX;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
LIVER;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	MALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
LIVER: (continued)									
Within Normal Limits	22	20	24	23	20	20	23	22	5
Vacuolation; hepatocellular	1	5	1	1	2	1	0	0	0
Necrosis; hepatocellular	0	0	0	0	0	0	0	1	0
Inflammation, mixed cell	2	0	0	1	2	3	2	2	0
Focus of cellular alteration, basophilic	0	0	0	0	1	0	0	1	0
Infarct	0	0	0	0	0	1	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	8
Hemangiosarcoma; malignant; secondary; incidental	0	0	0	0	0	0	0	0	0
LUNG;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	20	22	20	22	21	22	23	22	2
Hyperplasia; bronchioloalveolar; focal	1	0	3	0	0	0	0	1	3
Hemorrhage	0	0	0	0	0	0	0	0	0
Infiltration, histiocytic	0	0	0	1	1	0	0	0	0
Inflammation, mixed cell; focal	0	1	1	0	1	1	1	1	0
Mesothelioma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	0
Bronchioloalveolar carcinoma; malignant without metastasis; incidental	0	0	0	0	0	0	0	0	0
Bronchioloalveolar adenoma; benign; incidental	4	2	1	2	2	2	2	1	2
Lymphoma, malignant; malignant; secondary; incidental	0	1	0	0	0	0	0	0	8
Schwannoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
LYMPH NODE, MANDIBULAR;									
Examined	(25)	(25)	(24)	(25)	(23)	(23)	(25)	(25)	(13)
Within Normal Limits	25	25	24	25	23	23	25	25	10
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	1	0	0	0	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	1	0	1	2	0	0	0
Plasmacytosis; medullary sinus	0	0	0	0	0	0	0	0	0
Decreased cellularity; lymphoid	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	2

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- MALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
LYMPH NODE, MANDIBULAR; (continued)									
Schwannoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
LYMPH NODE, MESENTERIC;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	6
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	1
Decreased cellularity; lymphoid	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	6
MUSCLE, SKELETAL;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	9	9	4	13	14	11	12	6	5
Degeneration/regeneration; myofiber	16	16	21	12	11	14	13	19	2
Infiltration, mononuclear cell	0	0	0	0	0	1	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	6
NERVE, OPTIC;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
NERVE, SCIATIC;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	13
OVARY;									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-
Not Examined: NOT FOUND AT NECROPSY.	-	-	-	-	-	-	-	-	-
Cyst; paraovarian; unilateral	-	-	-	-	-	-	-	-	-

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- MALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
OVARY; (continued)									
Lymphoma, malignant; malignant; secondary; incidental	-	-	-	-	-	-	-	-	-
OVIDUCT;									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-
Not Examined: NOT FOUND AT NECROPSY.	-	-	-	-	-	-	-	-	-
Not Examined: NOT PRESENT IN SECTION.	-	-	-	-	-	-	-	-	-
Not Examined: NOT PRESENT IN WET TISSUES.	-	-	-	-	-	-	-	-	-
Lymphoma, malignant; malignant; secondary; incidental	-	-	-	-	-	-	-	-	-
PANCREAS;									
Examined	(25)	(25)	(25)	(25)	(25)	(24)	(25)	(25)	(13)
Within Normal Limits	25	25	25	24	25	24	25	25	13
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	1	0	0	0
Fibrosis; ductular	0	0	0	1	0	0	0	0	0
Dilatation; ductular	0	0	0	1	0	0	0	0	0
Inflammation, mixed cell; ductular	0	0	0	1	0	0	0	0	0
SKIN;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	24	25	25	24	25	8
Hemorrhage; subcutaneous tissue; regionally extensive	0	0	0	0	0	0	0	0	0
Hyperplasia; epidermal; focal	0	0	0	1	0	0	0	0	0
Crust; serocellular	0	0	0	1	0	0	0	0	0
Inflammation, mixed cell; dermal; focal	0	0	0	1	0	0	0	0	0
Dysplasia; adnexa	0	0	0	0	0	0	0	0	0
Cyst; follicle	0	0	0	0	0	0	0	0	0
Papilloma; squamous cell; benign; incidental	0	0	0	0	0	0	1	0	5
Schwannoma, malignant; malignant with metastasis; primary; fatal	0	0	0	0	0	0	0	0	1
SMALL INTESTINE, DUODENUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	MALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
SMALL INTESTINE, DUODENUM; (continued)									
Within Normal Limits	25	25	25	25	25	25	25	25	13
Not Examined: POSTMORTEM CHANGE PRECLUDES EVALUATION.	0	0	0	0	0	0	0	0	0
Sarcoma; not otherwise specified; malignant without metastasis; incidental	0	0	0	0	0	0	0	0	0
SMALL INTESTINE, ILEUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	13
Not Examined: POSTMORTEM CHANGE PRECLUDES EVALUATION.	0	0	0	0	0	0	0	0	0
SMALL INTESTINE, JEJUNUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	13
Not Examined: POSTMORTEM CHANGE PRECLUDES EVALUATION.	0	0	0	0	0	0	0	0	0
SPINAL CORD;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	12
Cyst; meninges	0	0	0	0	0	0	0	0	1
Cyst; squamous	0	0	0	0	0	0	0	0	0
SPLEEN;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	15	20	17	20	19	18	18	19	0
Pigment	0	0	0	0	0	0	0	0	0
Increased hematopoiesis	7	5	7	3	6	7	7	5	2
Atrophy	0	0	0	0	0	0	0	0	1
Hemorrhage; focal	0	0	0	0	0	0	0	0	1
Hemangiosarcoma; malignant with metastasis; primary; incidental	0	0	0	0	0	0	0	0	0
Hemangiosarcoma; malignant without metastasis; incidental	2	0	1	2	0	0	0	0	1

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- MALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
SPLEEN; (continued)									
Hemangiosarcoma; malignant without metastasis; fatal	1	0	0	0	0	0	0	1	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	10
STOMACH;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	24	24	25	25	25	0
Erosion; mucosal; glandular; focal	0	0	0	0	0	0	0	0	0
Infiltration, mixed cell	0	0	0	0	0	0	0	0	1
Infiltration, mixed cell; submucosal	0	0	0	0	0	0	0	0	0
Papilloma; multiple; squamous cell; benign; incidental	0	0	0	0	0	0	0	0	6
Papilloma; squamous cell; benign; incidental	0	0	0	1	1	0	0	0	7
Squamous cell carcinoma; malignant without metastasis; incidental	0	0	0	0	0	0	0	0	0
SUBCUTIS;									
Examined	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Hemangiosarcoma; malignant without metastasis; fatal	0	0	0	1	0	0	0	0	0
Schwannoma, malignant; malignant with metastasis; primary; incidental	0	0	0	0	0	0	0	0	0
TESTIS;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	24	25	25	25	13
Degeneration/atrophy; tubular; bilateral	0	0	0	0	1	0	0	0	0
THYMUS;									
Examined	(25)	(25)	(24)	(25)	(25)	(25)	(25)	(24)	(15)
Within Normal Limits	25	24	24	25	24	24	25	23	2
Not Examined: NOT PRESENT IN SECTION.	0	0	1	0	0	0	0	0	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	1	0
Hyperplasia; atypical	0	0	0	0	0	0	0	0	0

Provantis 9 - Production 03

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	MALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
THYMUS: (continued)									
Decreased cellularity; lymphoid	0	0	0	0	1	1	0	1	3
Lymphoma, malignant; malignant; secondary; incidental	0	1	0	0	0	0	0	0	9
Thymoma, malignant; malignant without metastasis; incidental	0	0	0	0	0	0	0	0	1
Mesothelioma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	0
Mesothelioma, malignant; malignant without metastasis; fatal	0	0	0	0	0	0	0	0	0
TONGUE:									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	13
TOOTH:									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Ameloblastoma; malignant without metastasis; fatal	0	0	0	0	0	0	0	0	0
TRACHEA:									
Examined	(25)	(25)	(25)	(25)	(24)	(25)	(25)	(24)	(13)
Within Normal Limits	25	25	25	25	24	25	25	24	13
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	1	0	0	1	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	0
URINARY BLADDER:									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(13)
Within Normal Limits	25	25	25	25	25	25	25	25	13
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	0
UTERUS:									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-
Not Examined: NOT PRESENT IN SECTION.	-	-	-	-	-	-	-	-	-
Cystic endometrial hyperplasia	-	-	-	-	-	-	-	-	-

Provantis 9 - Production 03

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	MALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
UTERUS: (continued)									
Anomaly; vascular; unilateral	-	-	-	-	-	-	-	-	-
Cyst; unilateral	-	-	-	-	-	-	-	-	-
Thrombus; vascular; unilateral	-	-	-	-	-	-	-	-	-
Schwannoma, malignant; malignant; secondary; incidental	-	-	-	-	-	-	-	-	-
Hemangiosarcoma; malignant without metastasis; incidental	-	-	-	-	-	-	-	-	-
Lymphoma, malignant; malignant; secondary; incidental	-	-	-	-	-	-	-	-	-
VAGINA:									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-

Provantis 8 - Production US

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	FEMALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
ARTERY, AORTA;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	24	25	25	25	4
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	1	0	0	0	7
Mesothelioma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
ARTERY, MESENTERIC;									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Thrombus	0	0	0	0	0	0	0	0	0
BODY CAVITY, NASAL;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
BODY CAVITY, ORAL;									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Squamous cell carcinoma; malignant without metastasis; fatal	0	0	0	0	0	0	0	0	0
BONE MARROW;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	24	7
Increased myeloid to erythroid ratio	0	0	0	0	0	0	0	1	0
Decreased cellularity	0	0	0	0	0	0	0	0	1
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	4
BONE, FEMUR;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
BONE, STERNUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	FEMALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
BONE, STERNUM; (continued)									
Within Normal Limits	25	25	25	25	25	25	25	25	12
BRAIN;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	10
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	2
CERVIX;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	23	25	25	25	10
Hemangiosarcoma; malignant without metastasis; fatal	0	0	0	0	1	0	0	0	0
Schwannoma, malignant; malignant; secondary; incidental	0	0	0	0	1	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	2
EAR;									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Inflammation, neutrophilic; tympanic cavity; bilateral	0	0	0	0	0	0	0	1	0
EPIDIDYMIS;									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-
Sperm granuloma; unilateral	-	-	-	-	-	-	-	-	-
Decreased cellularity; lumen; sperm; bilateral	-	-	-	-	-	-	-	-	-
Dilatation; ductular; focal	-	-	-	-	-	-	-	-	-
Lymphoma, malignant; malignant; secondary; incidental	-	-	-	-	-	-	-	-	-
ESOPHAGUS;									
Examined	(24)	(24)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	24	24	25	25	25	25	25	25	12

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- FEMALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
ESOPHAGUS; (continued)									
Not Examined: NOT PRESENT IN WET TISSUES.	1	1	0	0	0	0	0	0	0
Squamous cell carcinoma; malignant without metastasis; fatal	0	0	0	0	0	0	0	0	0
EYE;									
Examined.....	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits.....	25	25	25	25	25	25	25	25	0
Degeneration; retina; bilateral	0	0	0	0	0	0	0	0	11
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
GALLBLADDER;									
Examined.....	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(24)	(12)
Within Normal Limits.....	25	25	25	25	25	25	25	24	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	1	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	0
GALT;									
Examined.....	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(23)	(12)
Within Normal Limits.....	25	25	25	25	25	25	25	23	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	2	0
GLAND, ADRENAL;									
Examined.....	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits.....	1	2	2	2	2	9	8	10	3
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	0
Hyperplasia; subcapsular cell; bilateral	24	20	17	19	18	4	7	4	8
Hyperplasia; subcapsular cell; unilateral	0	3	6	4	5	11	10	11	1
Congestion; bilateral	0	0	0	0	0	1	0	0	0
GLAND, HARDERIAN;									
Examined.....	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits.....	25	24	21	22	24	24	23	25	9
Hyperplasia; unilateral; focal	0	0	2	0	0	0	0	0	1
Infiltration, mononuclear cell	0	0	2	3	0	0	2	0	0
Adenoma; benign; incidental	0	1	0	0	0	1	0	0	1

APPEARS THIS WAY ON ORIGINAL

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6f1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- FEMALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
GLAND, HARDERIAN; (continued)									
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	1	0	0	0	1
GLAND, LACRIMAL;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	24	25	23	22	23	12
Infiltration, mononuclear cell	0	0	0	1	0	2	3	2	0
GLAND, MAMMARY;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
GLAND, PARATHYROID;									
Examined	(9)	(10)	(16)	(14)	(15)	(17)	(13)	(18)	(3)
Within Normal Limits	8	9	15	13	15	16	12	17	3
Not Examined: NOT PRESENT IN SECTION.	16	15	9	11	10	8	12	7	9
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	0
Cyst; unilateral	1	1	1	1	0	1	1	1	0
GLAND, PITUITARY;									
Examined	(25)	(24)	(25)	(25)	(24)	(23)	(25)	(25)	(12)
Within Normal Limits	25	24	25	25	24	23	25	25	12
Not Examined: EXHAUSTED DURING HISTOLOGY PROCESSING.	0	0	0	0	0	0	0	0	0
Not Examined: LOST DURING NECROPSY.	0	0	0	0	0	1	0	0	0
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	1	1	0	0	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	1	0	0	0	0	0	0	0
GLAND, PROSTATE;									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-
Not Examined: NOT PRESENT IN SECTION.	-	-	-	-	-	-	-	-	-
Lymphoma, malignant; malignant; secondary; incidental	-	-	-	-	-	-	-	-	-

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- FEMALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
GLAND, SALIVARY;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	24	22	23	21	22	18	23	21	12
Infiltration, mononuclear cell	1	3	2	4	3	7	2	4	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	0
GLAND, SEMINAL VESICLE;									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-
Lymphoma, malignant; malignant; secondary; incidental	-	-	-	-	-	-	-	-	-
GLAND, THYROID;									
Examined	(25)	(25)	(25)	(25)	(25)	(24)	(24)	(25)	(11)
Within Normal Limits	25	25	25	25	25	24	24	25	11
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	1	1	0	1
GLAND, ZYMBALS;									
Examined	(24)	(25)	(23)	(23)	(24)	(22)	(23)	(23)	(10)
Within Normal Limits	24	25	23	23	24	22	23	23	10
Not Examined: NOT PRESENT IN SECTION.	1	0	2	2	1	3	2	2	2
HEART;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	24	24	25	25	9
Cardiomyopathy	0	0	0	0	0	0	0	0	1
Mesothelioma, malignant; malignant with metastasis ; primary; fatal	0	0	0	0	0	1	0	0	1
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	1	0	0	0	1
HEMOLYMPHORETICULAR TISSUE;									
Examined	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(12)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant with metastasis; primary; incidental	0	0	0	0	1	0	0	0	2

APPEARS THIS WAY ON ORIGINAL

Provantis 8 - Production 83

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- FEMALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
HEMOLYMPHORETICULAR TISSUE; (continued)									
Lymphoma, malignant; malignant with metastasis; primary; fatal	0	0	0	0	0	0	0	0	10
KIDNEY;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	22	23	23	24	19	21	24	20	6
Infiltration, mononuclear cell	1	1	2	0	3	3	1	2	0
Infiltration, mononuclear cell; pelvis	2	1	0	1	2	1	0	3	0
Degeneration/regeneration; tubular	0	0	0	0	1	0	0	0	0
Inflammation, mixed cell; multifocal	0	0	0	0	0	0	0	0	1
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	6
LARGE INTESTINE, CECUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	0
Single cell necrosis; mucosal	0	0	0	0	0	0	0	0	0
LARGE INTESTINE, COLON;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
LARGE INTESTINE, RECTUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
LARYNX;									
Examined	(25)	(25)	(25)	(24)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	24	25	25	25	25	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	1	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	0
LIVER;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	FEMALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
LIVER; (continued)									
Within Normal Limits	17	23	20	20	21	19	22	19	6
Vacuolation; hepatocellular	0	0	0	0	0	0	0	0	0
Necrosis; hepatocellular	0	0	1	0	0	0	0	0	0
Inflammation, mixed cell	8	2	4	5	4	5	3	6	0
Focus of cellular alteration, basophilic	0	0	0	0	0	1	0	0	0
Infarct	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	6
Hemangiosarcoma; malignant; secondary; incidental	0	0	1	0	0	0	0	0	0
LUNG;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	21	23	20	21	22	22	23	22	2
Hyperplasia; bronchioloalveolar; focal	1	0	1	0	1	0	1	2	2
Hemorrhage	0	0	0	0	0	0	0	0	1
Infiltration, histiocytic	2	1	0	0	0	0	0	1	0
Inflammation, mixed cell; focal	0	1	1	2	0	1	0	0	0
Mesothelioma, malignant; malignant; secondary; incidental	0	0	0	0	0	1	0	0	0
Bronchioloalveolar carcinoma; malignant without metastasis; incidental	0	0	0	0	0	1	1	0	0
Bronchioloalveolar adenoma; benign; incidental	1	0	3	2	1	1	0	1	2
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	1	0	0	0	9
Schwannoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	0
LYMPH NODE, MANDIBULAR;									
Examined	(25)	(24)	(25)	(25)	(25)	(24)	(25)	(24)	(11)
Within Normal Limits	24	23	25	25	24	24	25	24	4
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	1	0	1	1
Not Examined: NOT PRESENT IN WET TISSUES.	0	1	0	0	0	0	0	0	0
Plasmacytosis; medullary sinus	1	0	0	0	0	0	0	0	0
Decreased cellularity; lymphoid	0	1	0	0	1	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	7

APPEARS THIS WAY ON ORIGINAL

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic ---------- FEMALES ----------									
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
LYMPH NODE, MANDIBULAR; (continued)									
Schwannoma, malignant; malignant; secondary;									
incidental	0	0	0	0	0	0	0	0	0
LYMPH NODE, MESENTERIC;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	24	25	25	25	6
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	0
Decreased cellularity; lymphoid	0	0	0	0	1	0	0	0	0
Lymphoma, malignant; malignant; secondary;									
incidental	0	0	0	0	0	0	0	0	6
MUSCLE, SKELETAL;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	13	8	8	10	11	15	18	11	4
Degeneration/regeneration; myofiber	12	17	17	15	14	10	7	14	3
Infiltration, mononuclear cell	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary;									
incidental	0	0	0	0	0	0	0	0	6
NERVE, OPTIC;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(24)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	24	25	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	1	0	0
Lymphoma, malignant; malignant; secondary;									
incidental	0	0	0	0	0	0	0	0	0
NERVE, SCIATIC;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
OVARY;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(24)	(25)	(12)
Within Normal Limits	25	24	25	25	25	25	24	25	11
Not Examined: NOT FOUND AT NECROPSY.	0	0	0	0	0	0	1	0	0
Cyst; paraovarian; unilateral	0	1	0	0	0	0	0	0	0

APPEARS THIS WAY ON ORIGINAL

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	FEMALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
OVARY: (continued)									
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
OVIDUCT;									
Examined	(25)	(24)	(25)	(25)	(25)	(25)	(23)	(25)	(12)
Within Normal Limits	25	24	25	25	25	25	23	25	11
Not Examined: NOT FOUND AT NECROPSY.	0	0	0	0	0	0	1	0	0
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	1	0	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	1	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	1
PANCREAS;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	0
Fibrosis; ductular	0	0	0	0	0	0	0	0	0
Dilatation; ductular	0	0	0	0	0	0	0	0	0
Inflammation, mixed cell; ductular	0	0	0	0	0	0	0	0	0
SKIN;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	24	24	24	25	25	25	6
Hemorrhage; subcutaneous tissue; regionally extensive	0	0	1	0	1	0	0	0	0
Hyperplasia; epidermal; focal	0	0	0	0	0	0	0	0	1
Crust; serocellular	0	0	0	0	0	0	0	0	0
Inflammation, mixed cell; dermal; focal	0	0	0	0	0	0	0	0	0
Dysplasia; adnexa	0	0	0	1	0	0	0	0	0
Cyst; follicle	0	0	0	0	0	0	0	0	3
Papilloma: squamous cell; benign; incidental	0	0	0	0	0	0	0	0	3
Schwannoma, malignant; malignant with metastasis; primary; fatal	0	0	0	0	0	0	0	0	0
SMALL INTESTINE, DUODENUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(24)	(25)	(12)

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	FEMALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
SMALL INTESTINE, DUODENUM; (continued)									
Within Normal Limits	25	25	25	25	25	25	23	25	12
Not Examined: POSTMORTEM CHANGE PRECLUDES EVALUATION.	0	0	0	0	0	0	1	0	0
Sarcoma; not otherwise specified; malignant without metastasis; incidental	0	0	0	0	0	0	1	0	0
SMALL INTESTINE, ILEUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(24)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	24	25	12
Not Examined: POSTMORTEM CHANGE PRECLUDES EVALUATION.	0	0	0	0	0	0	1	0	0
SMALL INTESTINE, JEJUNUM;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(24)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	24	25	12
Not Examined: POSTMORTEM CHANGE PRECLUDES EVALUATION.	0	0	0	0	0	0	1	0	0
SPINAL CORD;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	24	25	25	25	25	24	22	12
Cyst; meninges	0	1	0	0	0	0	1	2	0
Cyst; squamous	0	0	0	0	0	0	0	1	0
SPLEEN;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	18	16	14	19	16	19	17	17	1
Pigment	1	0	0	0	0	0	1	0	0
Increased hematopoiesis	6	7	9	5	8	5	6	8	2
Atrophy	0	1	0	0	0	0	1	0	0
Hemorrhage; focal	0	0	0	0	0	0	0	0	0
Hemangiosarcoma; malignant with metastasis; primary; incidental	0	0	1	0	0	0	0	0	0
Hemangiosarcoma; malignant without metastasis; incidental	0	1	1	1	1	1	0	0	0

Provantis 8 - Production US

Appendix 15

Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	FEMALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
SPLEEN; (continued)									
Hemangiosarcoma; malignant without metastasis; fatal	0	0	0	0	0	0	0	0	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	9
STOMACH;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	23	25	24	25	25	25	25	25	1
Erosion; mucosal; glandular; focal	0	0	1	0	0	0	0	0	0
Infiltration, mixed cell	0	0	0	0	0	0	0	0	0
Infiltration, mixed cell; submucosal	1	0	0	0	0	0	0	0	0
Papilloma; multiple; squamous cell; benign; incidental	0	0	0	0	0	0	0	0	2
Papilloma; squamous cell; benign; incidental	1	0	0	0	0	0	0	0	9
Squamous cell carcinoma; malignant without metastasis; incidental	0	0	0	0	0	0	0	0	4
SUBCUTIS;									
Examined	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Hemangiosarcoma; malignant without metastasis; fatal	0	0	0	0	0	0	0	0	0
Schwannoma, malignant; malignant with metastasis; primary; incidental	0	0	0	0	1	0	0	0	0
TESTIS;									
Examined	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-	-	-	-	-
Degeneration/atrophy; tubular; bilateral	-	-	-	-	-	-	-	-	-
THYMUS;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
Within Normal Limits	25	24	23	25	22	22	23	22	2
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	0	0
Hyperplasia; atypical	0	0	0	0	0	0	1	0	0

APPEARS THIS WAY ON ORIGINAL

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	FEMALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
THYMUS; (continued)									
Decreased cellularity; lymphoid	0	1	1	0	2	2	1	2	0
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	1	0	0	0	11
Thymoma, malignant; malignant without metastasis; incidental	0	0	0	0	0	0	0	1	1
Mesothelioma, malignant; malignant; secondary; incidental	0	0	0	0	0	1	0	0	1
Mesothelioma, malignant; malignant without metastasis; fatal	0	0	1	0	0	0	0	0	0
TONGUE;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12
TOOTH;									
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0	0
Ameloblastoma; malignant without metastasis; fatal	0	0	0	0	0	0	0	0	1
TRACHEA;									
Examined	(25)	(24)	(25)	(25)	(25)	(24)	(25)	(25)	(12)
Within Normal Limits	25	24	25	25	25	24	25	25	12
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	1	0	0	0
Not Examined: NOT PRESENT IN WET TISSUES.	0	1	0	0	0	0	0	0	0
URINARY BLADDER;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(24)	(11)
Within Normal Limits	25	25	25	25	25	25	25	24	11
Not Examined: NOT PRESENT IN SECTION.	0	0	0	0	0	0	0	0	1
Not Examined: NOT PRESENT IN WET TISSUES.	0	0	0	0	0	0	0	1	0
UTERUS;									
Examined	(25)	(25)	(24)	(25)	(25)	(24)	(24)	(25)	(12)
Within Normal Limits	10	2	7	3	6	2	2	4	6
Not Examined: NOT PRESENT IN SECTION.	0	0	1	0	0	1	1	0	0
Cystic endometrial hyperplasia	15	23	16	22	18	22	22	21	4

Appendix 15 Pathology - Intergroup Comparison of Neoplastic Histo Pathology Observations

20105943 - A 26 week Carcinogenicity Study of Tenapanor and AZ13792925 by Oral Gavage in CByB6F1 Tg rasH2 Hemizygous mice

Observations: Neo-Plastic	FEMALES								
Removal Reasons: All of those SELECTED	0 mg/kg/	0 mg/kg/	0 mg/kg/	55 mg/kg/	110 mg/kg/	10/100 mg/kg/	30/300 mg/kg/	100/800 mg/kg/	75 mg/kg/
Number of Animals on Study :	25	25	25	25	25	25	25	25	15
Number of Animals Completed:	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(15)
UTERUS; (continued)									
Anomaly; vascular; unilateral	0	0	1	0	0	0	0	0	0
Cyst; unilateral	0	0	0	0	0	1	0	0	1
Thrombus; vascular; unilateral	0	0	0	0	0	0	0	0	1
Schwannoma, malignant; malignant; secondary; incidental	0	0	0	0	1	0	0	0	0
Hemangiosarcoma; malignant without metastasis; incidental	0	0	0	0	0	0	0	0	1
Lymphoma, malignant; malignant; secondary; incidental	0	0	0	0	0	0	0	0	3
VAGINA;									
Examined	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(12)
Within Normal Limits	25	25	25	25	25	25	25	25	12

Appendix 3: ExecCAC Meeting Minutes dated December 17, 2013 (RAT).

Executive CAC

Date of Meeting: December 17, 2013

Committee: David Jacobson-Kram, Ph.D., OND-IO, Chair
Abby Jacobs, Ph.D., OND-IO, Member
Paul Brown, Ph.D., OND-IO, Member
David Joseph, Ph.D., DGIEP, Alternate Member
Sushanta K. Chakder, Ph.D., DGIEP, Supervisor
Dinesh Gautam, Ph.D., DGIEP, Presenting Reviewer

Author of Draft: Dinesh Gautam, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The Committee did not address the sponsor's proposed statistical evaluation for the carcinogenicity bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND: 108,732
Drug Name: AZD1722 (RDX5791)
Sponsor: Ardelyx, Inc., Fremont, CA, USA

Background:
AZD1722, a sodium/hydrogen antiporter (NHE3) inhibitor, is being developed for the treatment (b) (4) constipation-predominant irritable bowel syndrome (IBS-C), (b) (4) The sponsor submitted a 2-year rat carcinogenicity study protocol and the rationale for dose selection for the proposed carcinogenicity study. The selection of the high dose for the proposed 2-year oral gavage carcinogenicity study was based on the toxicological findings of the 14-day, 1-month, 3-month and 6-month oral gavage toxicity studies with AZD1722 in rats.

Rat Carcinogenicity Study Protocol and Dose Selection
In the proposed 104-week carcinogenicity study in Crl:CD(SD) rats, groups of animals (60 rats/sex/group) will be orally (gavage) administered AZD1722 at dose levels of 0, 1.0, 3.0 and 10 mg/kg/day (dosing volume 1mL/kg/day). Control animals will receive the same volume of 0.1% Tween 80 (w/v) in deionized water (vehicle).

In the 14-day oral toxicity study in rats, RDX5791 was administered orally once daily at dose levels of 0, 0.1, 1.0, or 10 mg/kg/day. There were no mortalities in this study. Male rats in the 10

mg/kg/day dose group showed slightly lower body weight gain, a decrease in food intake and an increase in water consumption compared with control values. Microscopic findings revealed decreased goblet cells in the cecum, colon, and rectum of 0.1, 1.0, and 10 mg/kg/day groups. Most findings were reversed after a two-week recovery period. The NOAEL dose was 10 mg/kg/day for male and female rats.

The planned 1-month oral toxicity study with RDX5791 in rats was shortened to 2 weeks due to high mortality and other adverse effects. In this study, the animals were dosed with RDX5791 at 0, 30, 100, 300, and 1000 mg/kg/day for a period of 14 days. Treatment-related mortality was observed in all dose groups (1, 3, 3 and 4 rats died from 30, 100, 300 and 1000 mg/kg/day dose groups, respectively). Both male and female rats at all doses showed dramatic reductions of body weight on Day 14, ranging from 21% to 36%, compared to controls. The surviving rats showed several clinical and histopathological abnormalities.

In the 3-month oral toxicity study in rats, groups of animals were dosed with RDX5791 at 0, 0.1, 1.0 and 5.0 mg/kg/day. Test article-related clinical observations consisted of soft feces and brown staining of the anogenital and urogenital areas in the 1.0 and 5.0 mg/kg/day group males and females. No test article-related effects on body weights, food consumption, hematology, macroscopic parameters, serum chemistry, urinalysis parameters, organ weights, and microscopic findings were observed. The NOAEL dose was 5.0 mg/kg/day for both male and female rats.

In the 6-month ongoing oral toxicity study in rats, male and female rats were dosed with RDX5791 at 0, 0.1, 1.0 and 10.0 mg/kg/day. The Sponsor did not submit the study report in this submission; however, it was stated that 10 mg/kg/day was tolerated in this study. No significant treatment-related changes in the body weight gain were observed in males and females at any doses as compared to the control.

Executive CAC Recommendations and Conclusions:

- The Committee concurred with the Sponsor's proposed doses of 1.0, 3.0, and 10 mg/kg/day, by oral gavage, with the high dose based on mortality at 30 mg/kg/day in female rats, and body weight decrements at 30 mg/kg/day and higher doses in both male and female rats in the 28-day (shortened to 2 weeks) toxicity study.

- The Committee noted that the 2-year carcinogenicity study will be performed in a facility different from the one used in the 14-day, 1-month, 3-month and 6-month oral gavage toxicity studies. Exec-CAC concurrence for dose selection is contingent on there being no significant difference in toxicological findings through the first 6 months of dosing in the 2-year carcinogenicity study due to change of testing facility, such that dose recommendations would change.

If the Sponsor plans to conduct histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, they

will need to look at that tissue for all of the dose groups.
(b) for statistically significant or otherwise remarkable findings in the high dose group, the sponsor will need to look at the affected tissues in all of the dose groups.
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level, and
(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\

/Division File, DGIEP
/DGautam, DGIEP
/SChakder, DGIEP
/RPM/MScherer, DGIEP
/ASeifried, OND-IO

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

DAVID JACOBSON KRAM
12/19/2013

APPENDIX 4: Executive CAC Meeting minutes January 22, 2019

Executive CAC Final Study Minutes
Date of Meeting: January 22, 2019

Committee: Karen Davis Bruno, PhD, OND IO, Chair
Paul Brown, PhD, OND IO, Member
Tim McGovern, PhD, OND IO, Member
Ron Wange, PhD, OND IO, Member
Jay Chang, PhD, DAAAP, Alternate Member
Sushanta Chakder, PhD, DGIEP, Pharm/Tox Supervisor
Dinesh Gautam, PhD, DGIEP, Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations.

IND: 108732/NDA: 211801
Drug Name: Tenapanor (AZD1722/EDX5791) tablets
Sponsor: Ardelyx, Inc.

Background
Tenapanor (AZD1722 or RDX5791), a sodium (Na^+)/hydrogen (H^+) antiporter (NHE3) inhibitor, is being developed for the treatment of (b) (4) (b) (4) constipation-predominant irritable bowel syndrome (IBS-C), (b) (4) (b) (4) The Sponsor has submitted the final reports of the 2-year oral (gavage) carcinogenicity study of AZD1722 in rats and the 26-week oral carcinogenicity study of AZD1722, and AZ13792925 (major tenapanor metabolite) in Tg rasH2 mice. The doses used in the studies were concurred by the CDER Executive CAC.

Rat Carcinogenicity Study
Tenapanor dose levels used in the 2-year rat carcinogenicity study were 0 (vehicle, 0.1% Tween 80 in water), 1, 3, and 10 mg/kg/day. The high-dose of 10 mg/kg/day was selected based on the MTD from the 14-day and 6-month toxicology studies in rats. On Day 86, the 10 mg/kg/day dose level was reduced to 5 mg/kg/day due to a decrease in body weight up to 20%. On Day 92, the 3 mg/kg/day (mid-dose) dose level was reduced to 2 mg/kg/day for both male and female rats. Males in the high-dose group (10/5 mg/kg/day) were terminated early at Week 83, as only 15 animals survived in the group. All other male groups were terminated at Week 86 after only 20 animals survived in the male vehicle control group. All female groups were terminated on Week 96 due to survival in the vehicle control female group reaching 20 animals.

There were no tenapanor-related significant effects on survival of male or female rats. Tenapanor-treated animals showed up to 20% reduction in body weight within the first 3 months of the study. Treatment with tenapanor was not associated with a treatment-related significant increase in neoplasms in male and female rats.

Mouse Carcinogenicity Study
In the 26-week carcinogenicity study in Tg rasH2 mice with tenapanor and its major metabolite, AZ13792915, tenapanor doses of 100, 300 and 800 mg/kg/day were used for females, and 10, 30

and 100 mg/kg/day for males. The doses of AZ13792915 used were 55 and 165 mg/kg/day for both male and female mice. The control groups received the vehicles (0.1% Polysorbate 80, 1% DMSO in Milli-Q Water for tenapanor; 0.1% Polysorbate 80 in Milli-Q Water for AZ13792925). The positive control was 7.5 mg/mL N-Nitrosomethylurea (NMU) in Citrate-buffered saline (pH 4.5).

There were no tenapanor- or AZ13792925-related effects on survival rates of male or female Tg rasH2 mice. For the positive control group, there was a high incidence of mortality in males (13 out of 15) and females (12 out of 15). Males and females in the 165 mg/kg/day AZ13792925 dose group showed reductions of body weight (13.3% and 8.3%, respectively), compared to controls. Due to the decrease in mean body weight, the high-dose of 165 mg/kg was reduced to 110 mg/kg/day on Day 99. The body weight was lower in males (12.8%) and females (7.0%) at study termination day. No tenapanor- or AZ13792925-related neoplastic or non-neoplastic findings were noted in male and female Tg rasH2 mice. The positive control group exhibited the expected neoplastic changes.

Executive CAC Conclusions

Rat Carcinogenicity

- The Committee concurred that the carcinogenicity study conduct was adequate, noting prior approval of the protocol.
- The Committee concurred that there were no drug-related neoplasms in the 2-year rat carcinogenicity study in either males or females.

Mouse Carcinogenicity

- The Committee concurred that the carcinogenicity study conduct was adequate, noting prior approval of the protocol.
- The Committee concurred that there were no drug-related neoplasms in the 26-week carcinogenicity study in either male or female Tg rasH2 mice.

Karen Davis Bruno, PhD
Chair, Executive CAC

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

DINESH C GAUTAM
02/13/2019 04:05:45 PM

SUSHANTA K CHAKDER
02/13/2019 04:10:36 PM