CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214662Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Administrative Application Information

Category	Application Information
Application type	NDA
Application number(s)	214662
Priority or standard	Priority
Submit date(s)	1/29/2021
Received date(s)	1/29/2021
PDUFA goal date	9/29/2021
Division/office	Division of Hepatology and Nutrition (DHN)
Review completion date	9/23/2021
Established/proper name	Maralixibat
(Proposed) proprietary name	Livmarli
Pharmacologic class	Ileal bile acid transporter (IBAT) inhibitor
Code name	Cholestatic liver diseases (i.e., primary biliary cirrhosis and primary sclerosing cholangitis) (7060106)
Applicant	Mirum Pharmaceuticals, Inc.
Dosage form(s)/formulation(s)	Liquid formulation
Dosing regimen	380 mcg/kg/day
Applicant proposed indication(s)/ population(s)	Treatment of cholestatic pruritus in patients with Alagille syndrome 1 year of age and older
Proposed SNOMED indication	Alagille syndrome (code 31742004 arteriohepatic dysplasia disorder)
Regulatory action	Approval
Approved dosage (if applicable)	See above Dosing regimen
Approved indication(s)/ population(s) (if applicable)	See above indication(s)/populations(s)
Approved SNOMED term for indication (if applicable)	See above Proposed SNOMED indication

Abbreviations: PDUFA, Prescription Drug User Fee Act; SNOMED, Systematized Nomenclature of Medicine

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Glossary

AE	adverse event
AESI	adverse event of special interest
ALGS	Alagille syndrome
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ARW	after randomized withdrawal
ASBT	apical sodium-dependent bile acid transporter
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
$AUC_{0\text{-}24h}$	area under the concentration-time curve from time 0 to 24 h
AUC_{last}	area under the concentration-time curve to the last measurable concentration
BA	bile acids/bioavailability
BCRP	breast cancer resistance protein
BID	twice daily
BMC	bone mineral content
BMD	bone mineral density
CDS	clinical data scientist
CI	confidence interval
CIC	caregiver impression of itch
C_{max}	maximum plasma concentration
CYP	cytochrome P450
DPMH	Division of Pediatric and Maternal Health
EAP	expanded access program
ECAC	Executive Carcinogenicity Assessment Committee
ECHA	European Chemicals Agency
ET	end of treatment
fBA	fecal bile acids
FDA	Food and Drug Administration
FGF	fibroblast growth factor
FMQ	Food and Drug Administration Medical Dictionary for Regulatory Activities query
FPE	first-pass effect
FSV	fat-soluble vitamin
GD	gestation day
GI	gastrointestinal
GGT	gamma glutamyl transferase
IBAT	intestinal bile acid transporter
IC_{50}	half-maximal inhibitory concentration
ICH	International Council on Harmonisation
IND	investigational new drug
INR	international normalized ratio
IR	information request

ISS	integrated summary of safety
ItchRO(Obs)	Itch Reported Outcome (Observer)
ItchRO(Pt)	Itch Reported Outcome (Patient)
IV	intravenous
LD	lactation day
LLOQ	lower limit of quantitation
LS	least squares
LSC	liquid scintillation counting
LTE	long-term extension
MNU	N-methyl-N-nitrosourea
NDA	new drug application
NOAEL	no observable adverse effect level
OATP	organic anion transporting polypeptide
OL	open-label
OLE	open-label extension
pBDL	partial bile duct ligation
PBPK	population-based pharmacokinetics
PD	pharmacodynamic
PDE	permitted daily exposure
PI	Prescribing Information
PK	pharmacokinetic
PLB	placebo
PND	postnatal day
PT	preferred term
QC	quality control
QD	once daily
(Q)SAR	quantitative structure-activity relationship
RD	risk difference
RWD	randomized withdrawal
SAE	serious adverse event
SAP	statistical analysis plan
SE	standard error
SIAP	statistical interim analysis plan
sBA	serum bile acids
SD	standard deviation
TBM	to-be-marketed
TEAE	treatment-emergent adverse event
TK	toxicokinetic
T_{max}	time to maximum concentration
UDCA	ursodeoxycholic acid
ULN	upper limit of normal

I. Executive Summary

1. Summary of Regulatory Action

Maralixibat (Livmarli®) is being approved for the treatment of pruritus in patients with Alagille syndrome (ALGS). Pruritus that can be relentless and severely debilitating is the hallmark clinical symptom of this disease. Intractable pruritus is a reason for liver transplantation for some patients regardless of liver disease severity. Surgical biliary diversion procedures are also done for patients with intractable pruritus. There are no Food and Drug Administration (Agency)-approved therapies for treatment of pruritus in patients with ALGS. This is an area of unmet medical need in a rare disease with significant morbidity.

One multicenter adequate and placebo-controlled randomized withdrawal study of maralixibat, Study LUM001-304, showed a direct measurement of benefit for treatment of pruritus in patients with ALGS. A parent or caregiver observational assessment of pruritus was used as a primary efficacy measure of how a patient feels or functions. The Applicant satisfactorily evaluated the qualitative characteristics of an observer-reported outcome instrument, the Itch Reported Outcome (Observer) (ItchRO(Obs)), which was used to calculate the primary efficacy endpoint in quantitative analyses. The Agency's main analysis of efficacy was based on endpoints evaluating weekly averages of the worst daily scores of the ItchRO(Obs). Due to the Applicant's choice of serum bile acids (sBA) measurements as a primary endpoint and the lack of prespecification of a single analysis for the pruritus endpoint, the Agency evaluated the ItchRO(Obs) results using different analyses, such as, mean change in scores from Week 18 to Week 22 and mean change in scores from baseline to Week 22. These analyses demonstrated a consistent benefit of continuing maralixibat for 22 weeks, when compared with switching to placebo after Week 18. Supportive analyses were conducted on the basis of the patient-reported outcome, Itch Reported Outcome (Patient), in patients who were old enough and able to self-report their itching.

The statutory standard of substantial evidence of effectiveness was met with this single study for the following reasons: (1) statistically persuasive findings on the primary pruritus endpoint analysis and on supportive analyses of the observer endpoints; (2) consistent benefit across centers; and (3) post hoc analyses demonstrating consistent findings between observer- and patient-reported outcomes on pruritus endpoints.

Supportive evidence for the effectiveness of maralixibat is provided by the following: (1) reduction in sBA levels (elevated sBA levels are hypothesized to be related to pruritus); (2) on average, a return to baseline levels of pruritus was mirrored by a return to baseline sBA levels in the placebo arm; and (3) nonclinical proof-of-concept studies to support the mechanistic rationale for treatment of pruritus with maralixibat in patients with ALGS.

The safety information supports a favorable benefit/risk assessment. Maralixibat is minimally absorbed with low systemic exposure in patients at the recommended doses. Gastrointestinal adverse reactions such as diarrhea, abdominal pain, and vomiting were observed but can be addressed by treatment interruption or treatment discontinuation. No differences were observed

in serious adverse events compared to placebo in the pivotal trial; however, a 4-week randomized withdrawal placebo-controlled period is limited in its ability to detect any differences. Two supportive placebo-controlled studies of 13-weeks duration assessed doses less than 400mcg/kg/day and a majority of exposure to maralixibat occurred in an open-label, uncontrolled studies.

Healthy volunteer studies with maralixibat demonstrated mild elevations in alanine aminotransferase and animal toxicology studies demonstrated fat-soluble vitamin (FSV) deficiency at higher doses. Treatment-emergent liver test abnormalities, FSV deficiency and bone fractures were observed in open-label portions of the pivotal trial and in supportive studies. However, these adverse events commonly occur in ALGS (e.g., the presence of FSV deficiency is one criterion that can support the diagnosis of ALGS). Therefore, the extent to which maralixibat may affect the frequency and severity of these events is unclear. Careful review of liver test abnormalities, for example, suggests that most are unrelated to maralixibat, although several are potentially related to maralixibat exposure, with improvement after drug discontinuation or decrease in dose. These observations of liver test abnormalities, gastrointestinal adverse reactions, and FSV deficiency are included in the Warnings and Precautions to alert clinicians to carefully follow patients who experience these adverse reactions.

To address concerns regarding the limited safety data available, the Sponsor will provide a final study report on the safety experience of patients in an ongoing open-label trial (MRX-800). In addition, a 5-year registry-based study will collect data on the health outcomes of patients chronically treated with maralixibat. This study will report yearly on incidence of surgery (biliary diversion surgery and liver transplantation), all-cause mortality, assessment of growth and incidence of fat-soluble vitamin deficiencies and their long-term sequelae (e.g., bone fractures, bleeding episodes).

A dose of 400 mcg/kg/day (380 mcg/kg/day free base equivalent) is the only dose that demonstrates persuasive findings of benefit on pruritus. It was the only dose evaluated in the pivotal trial in a placebo-controlled manner. Lower doses (up to 280 mcg/kg/day) in supportive studies did not demonstrate efficacy on pruritus symptoms. In the pivotal trial the dose was gradually increased over a 6-week period from 14 mcg/kg/day to 400 mcg/kg/day, with the intent to minimize gastrointestinal (GI) (i.e., diarrhea, abdominal pain, vomiting) side effects of maralaxibat. However, experience from an expanded access program (EAP) demonstrated that a more rapid dose escalation may also be well tolerated. EAP participants were dosed with 200 mcg/kg/day (190 mcg/kg/day free base equivalent) for the first 7 days followed by 400 mcg/kg/day. Data were submitted for 24 participants (22 were treatment-naïve): 2 patients had mild GI adverse effects, neither had to pause or discontinue treatment. Based on demonstrated tolerance with a more rapid dose escalation, and absence of demonstrated benefit at lower doses, we have approved the 7-day dose escalation approach.

Approval for use in patients >18 years of age will be recommended. No adult patients were enrolled. However, the pathophysiology and the mechanism of action of the drug are the same for children and adults. Adult patients with ALGS who either present with new onset pruritus secondary to ALGS or responded to treatment as children and have turned 18 years or older would benefit from access to treatment. Due to the extreme rarity of the disease in adults (about one in four will survive to adulthood without a liver transplant), it will be impracticable if not

impossible to conduct a trial in adults. Therefore, the drug will be approved for patients age one and older.

The Applicant was granted a rare pediatric disease designation from the Office of Orphan Products Development and the Office of Pediatric Therapeutics and met all criteria for receiving a rare pediatric disease priority review voucher.

In summary, our review of the Applicant's NDA demonstrated that maralixibat is safe and effective for the treatment of cholestatic pruritus in patients with ALGS.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	Alagille syndrome (ALGS) is a rare (incidence of 1 in 30,000 to 1 in 70,000), autosomal dominant, multi-organ disease. Bile duct paucity in the liver occurs in approximately 90% of patients with ALGS. Bile duct paucity leads to impaired bile flow and subsequent cholestatic liver injury, which manifests as jaundice and pruritus. The disease presents during early childhood with pruritus, elevated liver tests, jaundice, and growth failure. The disease can progress to cirrhosis leading to liver failure. A majority who present with cholestasis early in life will require a liver transplant before reaching adulthood. Pruritus is a severe and disabling symptom in patients with ALGS. The physical manifestations range from scratch marks, excoriations, and scarring due to persistent and unrelenting pruritus.	ALGS is a rare, serious condition presenting in childhood associated with severe pruritus. The itching and scratching are disabling symptoms and can be disruptive for patients and families, and limit patients' ability to participate in daily activities.
Current Treatment Options	Off-label medical treatment for pruritus includes use of ursodeoxycholic acid (UDCA), cholestyramine, antihistamines, naltrexone, rifampin, and ondansetron. Most of these medical therapies do not ameliorate pruritus and some of these therapies have undesirable adverse events (AEs). When the pruritus is severe, and quality of life is significantly impacted, patients undergo surgical intervention. Surgical interventions include internal or external biliary diversion. Severe intractable pruritus, regardless of the actual disease process in the liver, is considered an indication for liver transplantation. Complications of a liver transplantation procedure are serious. Moreover, after liver transplantation, patients must be maintained on lifelong immunosuppression, which has its own complications and associated serious adverse events.	There is no U.S. Food and Drug Administration (FDA)-approved medical therapy for treatment of pruritus in patients with ALGS. A majority of patients do not respond to the available off-label medical treatments, and patients continue to experience intractable pruritus. Surgical options (biliary diversion and liver transplantation) are invasive and associated with numerous serious adverse events. Treatment of pruritus in patients with ALGS remains a high unmet medical need.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	Study LUM001-304 was designed as a Phase 2 study and enrolled 31 patients with ALGS in an open-label study with a 4-week, double-blind, placebo-controlled, randomized withdrawal period (RWD). Twenty-nine patients completed the open-label run-in period and were randomized to the RWD period as follows: Placebo arm N=16; maralixibat 400 mcg/kg arm N=13. Efficacy measures of pruritus used for Study LUM001-304 included itch observer-reported outcome (ItchRO[Obs]) and patient-reported outcome (ItchRO[Pt]) assessments. Patients' pruritus was assessed by their caregiver twice daily (BID) on a single-item ItchRO(Obs) with scores ranging from 0 (none observed or reported) to 4 (very severe). Patients' pruritus was assessed BID on a single-item ItchRO(Pt) with scores ranging from 0 ("I didn't feel itchy") to 4 ("I felt very, very itchy"). Independent self-reporting of itching severity was limited to older patients. A total of 14 patients completed the ItchRO(Pt) either on their own or with the help of their caregiver. Of these, seven patients (four in the placebo arm and three in the maralixibat arm) completed the ItchRO(Pt) independently. There was a high concordance between the ItchRO(Obs) and ItchRO(Pt) pruritus scores in these seven patients. *Primary Efficacy Endpoint* The primary endpoint specified in the protocol was based on the mean change in serum bile acids (sBAs). However, an effect on sBAs is not a validated surrogate endpoint that can be used to demonstrate clinical benefit. Effects on pruritus were secondary endpoints without a prespecified single primary pruritus endpoint. A decrease in pruritus scores was observed in the open-label run in period; however, this was not used in the primary analysis due to the lack of a comparator arm and the potential for bias in observer-reported and patient-reported outcomes when caregivers	The clinical study design and FDA analyses were adequate for evaluation of pruritus in the ALGS population, a disabling symptom of a rare disease for which there are currently no FDA approved treatment options for people with ALGS. On average, patients who continued treatment with maralixibat had improved pruritus compared to patients who were switched to placebo. Those who were randomized to placebo had a return of pruritus symptoms similar to their baseline symptoms. Reduction in pruritus is clinically meaningful. In addition, analyses were also performed using different summaries of the daily pruritus scores (e.g., morning scores, evening scores, average of the two daily scores). The results of these analyses were consistent with the FDA's primary efficacy assessment. Study LUM001-304 did not enroll adults. The FDA decided to approve maralixibat for patients with ALGS aged 1 year and older. This decision was based on unmet medical need, to allow access to adult patients with ALGS. The biology of the disease is similar and mechanism of action of drug is also the same across all age groups. The mechanism of ileal bile acid transporter (IBAT) inhibitors would allow reduction in pruritus across all patients with ALGS. The pathophysiology of pruritus is not completely understood and is considered multifactorial. While the complete mechanism for pruritus is not fully understood, elevated sBA levels may be associated with pruritus in patients with cholestatic liver diseases. In the clinical study, patients with ALGS with elevated serum bile acid levels were enrolled and sBA levels were reduced after maralixibat treatment. As such, the reduction in sBA levels as a result of reduced reabsorption of bile salts may provide indirect supportive

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	and patients are aware that the patient is receiving active treatment. The FDA focused on the 4-week RWD period comparing those who remained on maralixibat with those who switched to placebo. In the absence of a single pruritus endpoint, the Agency analyzed several pruritus endpoints, which consistently demonstrated a benefit of continuing maralixibat treatment over withdrawal to placebo after 18 weeks of open-label treatment. Two of these analyses are presented here. Using the 0 (no pruritus) to 4 (severe pruritus) scale, the mean (95% confidence interval [CI]) of patients' weekly average of the worst daily ItchRO(Obs) score at Week 22 for maralixibat was 1.6 (1.1, 2.1) and for placebo was 3.0 (2.6, 3.5). The mean (95% CI) change from Week 18 to Week 22 in the weekly average of the worst daily ItchRO(Obs) score for maralixibat was 0.2 (-0.3, 0.7) and placebo was 1.6 (1.2, 2.1), with a mean (95% CI) treatment difference of -1.4 (-2.1, -0.8). After the RWD, patients in the placebo arm resumed maralixibat treatment. On average, these patients returned to the same level of pruritus improvement observed in the patients randomized to maralixibat in the RWD period. *Pharmacodynamic Effects* Study LUM001-304 enrolled patients with elevated sBA levels (>3× the upper limit of normal [ULN] for age). Maralixibat treatment lowered sBA levels during the open-label run-in period. During the RWD period, the average sBA level in the placebo arm returned to the baseline average sBA level. Although on average, treatment with maralixibat led to decreased sBA levels and improvement in pruritus symptoms, there was not a clear association at the individual patient level between the magnitude of the decrease of sBA and the magnitude of pruritus	evidence of mechanism of action. sBA level is distal from disease course and clinical outcomes, i.e., sBA levels do not provide an accurate estimation of hepatocellular bile acid levels, which are the key mediators of liver damage.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	improvement. A decrease in sBA levels has not been established as a biomarker that predicts improvement in pruritus.	
Risk and Risk Management	The safety database consisted of 86 patients (31 patients enrolled in LUM001-304 and an additional 55 patients enrolled in supportive studies using lower doses of maralixibat). Exposure occurred in a predominantly open-label manner without placebo control (for example, the 4-week RWD period is the only placebo-controlled exposure in LUM001-304). The median duration of exposure in the safety database was 32.3 months, most of this exposure in the absence of placebo control. Liver test abnormalities, fat-soluble vitamin (FSV) deficiency among other adverse events (e.g., bone fractures) observed in the marlaxibat studies are also established complications of ALGS. In light of the limited placebo-control followup, rates of common adverse events (AEs) are described while patients were exposed to maralixibat (without a placebo comparator). The most common adverse events included the following: *Liver Test Abnormalities* In the overall development program, elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were observed in 16/86 (18.6%) patients. Seven (8.1%) discontinued maralixibat and three (3.5%) had a decrease in dose or dose interruption in response to ALT elevations. In LUM001-304, ALT elevations occured in 4/31 (12.9%) during the open-label extension (OLE) period, which led to dose modification, interruption, or discontinuation.	The absence of long-term placebo control and the presence of liver test abnormalities and FSV deficiency as known manifestations of ALGS, make it difficult to determine the effect of maralixibat exposure on the frequency and severity of these adverse events. *Liver Test Abnormalities* A small safety database in the absence of placebo control limits assessment of liver test abnormalities. These AEs of liver test abnormality can be monitored; i.e., liver tests should be obtained at baseline and monitored frequently while maralixibat is administered. Treatment interruption, dose reduction, or treatment discontinuation is recommended based on the seriousness of the liver test abnormalities or if there is evidence of hepatic decompensation. *Gastrointestinal (Diarrhea, Abdominal Pain, Vomiting) AEs* Gastrointestinal AEs are monitorable and can be managed based on symptoms. Treatment interruption, dose reduction, or treatment discontinuation is recommended, based on the seriousness of the gastrointestinal symptoms. If diarrhea or vomiting lead to dehydration, are persistent, or require intervention (e.g., intravenous hydration), treatment with maralixibat should be interrupted. Maralixibat can be restarted at 190 mcg/kg and increased to 380 mcg/kg as tolerated. *Fat-Soluble Vitamin Deficiency* FSV levels should be obtained at baseline and monitored frequently while patients are administered maralixibat. If patients develop FSV deficiency, FSV should be supplemented. If patients develop clinical symptoms of FSV deficiency and the FSV deficiency is unresponsive to

	Elevations in total/direct bilirubin were observed in four (4.7%) patients. Two patients discontinued maralixibat in response to bilirubin elevations. One patient discontinued treatment due to bilirubin elevation in the first 48 weeks of treatment LUM001-304. Data from healthy volunteer studies demonstrated mild elevations in ALT, indicating a potential signal, even in the absence of underlying liver disease. Five patients underwent liver transplant during open-label extension (median duration of exposure of 32 months), including three for liver disease progression occurring several years after starting treatment with maralixibat. However, this rate of liver transplant is not unexpected in light of the natural history of ALGS. *Gastrointestinal AEs (Diarrhea, Abdominal Pain, Vomiting)* GI AEs, specifically diarrhea (n=41, 47.8%), abdominal pain (n=40, 46.5%) and vomiting (n=32, 37.2%), were the most common AEs, sometimes occuring together. Five (6%) patients required dose interruptions and interventions (e.g., intravenous hydration, observation in the hospital) for these symptoms. *Fat-Soluble Vitamin Deficiency* Among the patients, 22 of 86 (25.6%) had an AE of FSV deficiency, such as international normalized ratio elevation or vitamin D deficiency. FSV deficiency is an established complication of cholestatic liver disease, including ALGS, and a significant portion of patients were taking FSV supplements at study entry. In addition, nonclinical (animal) toxicology studies of maralixibat demonstrated FSV deficiency, including bleeding related to vitamin K deficiency, indicating that maralixibat may also have an effect on FSV deficiency.	supplementation, based on severity of symptoms, consider treatment interruption, dose reduction or treatment discontinuation.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	Among the patients, 3/31 (9.7%) developed FSV deficiency in the first 48 weeks of treatment in LUM001-304. Overall, 22/86 (25.6%) patients developed FSV deficiency.	

2.2. Conclusions Regarding Benefit-Risk

ALGS is a rare, pediatric cholestatic liver disease, in which pruritus is a debilitating symptom. Patients with ALGS with intractable pruritus who do not respond to the current (off-label) standard-of-care treatments have no therapeutic options for symptomatic relief of pruritus. Patients with pruritus who are refractory to medical therapy undergo surgical biliary diversion or liver transplantation. Both procedures are invasive, specifically liver transplantation, for which life-long immunosuppression is required and is associated with complications related to immunosuppression. This population needs new and effective treatment that could reduce pruritus. Maralixibat is an intestinal bile acid transport (IBAT) inhibitor that was granted Breakthrough Therapy designation for drug development as well as a Rare Pediatric Disease Designation for the treatment of pruritus associated with ALGS.

Patients with ALGS who had moderate to severe pruritus at baseline had an observed improvement in pruritus scores after receiving maralixibat 380 mcg/kg/day. The pivotal study, Study LUM001-304, demonstrated the superiority of maralixibat compared to placebo in maintaining the improvement in pruritus scores over a 4-week blinded, randomized, treatment withdrawal period.

The Agency has decided to approve a dose of 380 mcg/kg/day of maralixibat (equivalent to 400 mcg/kg/day of maralixibat chloride). The recommended starting dose of 190 mcg/kg (equivalent to 200 mcg/kg/day of maralixibat chloride) for one week is designed to minimize gastrointestinal adverse events that would limit tolerance and lead to treatment interruption or discontinuation. The maximum daily dose of maralixibat that can be administered to patients is 28.5 mg.

The safety of maralixibat long-term use is not well characterized. In the context of a rare disease the safety data for maralixibat is acceptable for the proposed dosing regimen in the intended population for the treatment of pruritus. Overall, maralixibat has a favorable safety profile and the safety concerns can be adequately addressed in the labeling. The nature and frequency of the safety events (liver test abnormalities, diarrhea/abdominal pain/vomiting, and potential fat-soluble vitamin deficiency) can be mitigated in the target population by monitoring for adverse events. There are no current treatment options for this population.

Based upon review of all the available efficacy and safety data, the benefit of maralixibat outweighs the risk of treatment in patients with ALGS, who have medically refractory pruritus. The availability of maralixibat will provide a new and effective treatment option for this patient population.

Table 3. Benefit-Risk Effects

Effect	Maralixibat 400 mcg/kg N=13	Placebo N=16	Mean Treatment Difference	Uncertainties
Benefits (Favorable Effects)				
Pruritus at the end of the RWD period,[a] mean (95% CI)				For these endpoints, remaining on maralixibat 400 mcg/kg during the RWD period resulted in a beneficial effect on pruritus compared to switching to placebo. Supportive studies of doses up to 280 mcg/kg did not demonstrate superiority to placebo. There remains uncertainty as to the magnitude of benefit on pruritus upon initiation of maralixibat 400 mcg/kg, as the study design had an initial open-label treatment period with no control arm.
Week 22 mean	1.6 (1.1, 2.1)	3.0 (2.6, 3.5)	-1.4 (-2.1,-0.8)	
Change from Week 18 to Week 22	0.2 (-0.3, 0.7)	1.6 (1.2, 2.1)	-1.4 (-2.1,-0.8)	
Risks (Unfavorable Effects)				
Abnormality of liver tests, n (%)	0 (0)	0 (0)		A 4-week placebo control period after an 18-week open-label run-in period is inadequate to detect a difference between the two arms in potential safety events. Multiple events occurred during open-label treatment. The clinical impact of liver test elevations over time with chronic dose administration is uncertain.
Fat-soluble vitamin deficiency, n (%)	0 (0)	0 (0)		A 4-week placebo control period after an 18-week open-label run-in period is inadequate to detect a difference between the two arms for potential safety events. The clinical impact of vitamin deficiency is uncertain but could include effects on cognition, growth, and bone fractures and development.
Gastrointestinal symptoms, n (%)				A 4-week placebo control period after an 18-week open-label run-in period is inadequate to detect a difference between the two arms in potential safety events.
Diarrhea	1 (7.7)	1 (6.2)		
Abdominal pain	1 (7.7)	1 (6.2)		
Vomiting	1 (7.7)	1 (6.2)		

[a] Patients' pruritus was assessed by their caregiver twice daily on a single-item ItchRO(Obs) assessment with scores ranging from 0 (no scratching) to 4 (worst possible scratching).The average of the worst daily ItchRO(Obs) scores was computed for each week. The average of this outcome across patients in each treatment group was analyzed using an ANCOVA model adjusted for the Week-18 ItchRO(Obs) score.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); RWD, randomized withdrawal

II. Interdisciplinary Assessment

3. Introduction

Mirum seeks approval of Livmarli (maralixibat) for the "treatment of cholestatic pruritus in patients with Alagille syndrome (ALGS)." The proposed recommended dose is 400 mcg/kg/day (380 mcg/kg/day free-base equivalent), as an oral solution.

Maralixibat is an inhibitor of the ileal bile acid transporter (IBAT). Bile acids (BAs) are synthesized from cholesterol in the liver. The primary function of BAs is to solubilize lipids into micelles, aiding digestion and absorption of fat and fat-soluble vitamins (FSV). Approximately 95% of the BAs secreted in the lumen of the gastrointestinal (GI) tract are reabsorbed in the terminal ileum via IBAT. Inhibition of IBAT inhibits the re-absorption of BAs in the terminal ileum, thereby interfering with the enterohepatic circulation and decreasing the serum BA pool. Reducing BA is proposed as an approach to treat cholestatic pruritus.

ALGS is rare (prevalence of 1:30,000 to 1:70,000 (Danks et al. 1977; Leonard et al. 2014), but is the most common cause of inherited cholestasis in children. Although hepatic involvement is present in most patients with ALGS, it is a multisystem disorder. The natural history of ALGS is variable due to differences in end-organ involvement. However, cholestasis usually presents in the first 3 months and pruritus in the first year of life. ALGS is associated with a poor quality of life and significantly reduced survival in those who present with cholestasis. In a series of 377 patients with ALGS, the liver-transplant-free survival rate at age 18.5 years was 24% (Kamath et al. 2020).

Clinical Features

ALGS presents with clinical features of chronic cholestasis, cardiovascular abnormalities, butterfly vertebrae, posterior embryotoxon, renal anomalies, vascular abnormalities, and characteristic facies. The phenotypic expression of the disease is variable and comprises individuals with minimal phenotypic evidence of the disease to others who have end-stage liver disease, require liver transplantation, and die of liver failure, cardiac disease, or vascular catastrophes. However, a common feature of ALGS is severe cholestasis and associated unremitting pruritus (Turnpenny and Ellard 2012). Cholestatic pruritus is associated with a significantly negative impact on quality of life, causes sleep deprivation resulting in fatigue, and exerts negative effects on mood, such as suicidal ideation. Due to these negative effects on affected patients, pruritus is an indication for liver transplantation, even in the absence of liver failure (Düll and Kremer 2020).

Diagnosis

The diagnosis is made on the basis of both genetic testing and clinical parameters. Mutations/deletions in two genes associated with the Notch signaling pathways are known to cause ALGS: JAGGED1 (in approximately 90% of ALGS cases) and NOTCH2 in a small minority of ALGS cases. Although most patients are diagnosed during the first year of life, the

age of presentation ranges from 16 weeks to 10 years. The most common presenting signs are jaundice and pruritus, occurring in 80% of patients Pruritus occurs earlier in patients who had neonatal jaundice than in patients who are anicteric (Lykavieris et al. 2001). The hepatic manifestations of ALGS consist of cholestasis, bile duct paucity, cirrhosis, hypercholesterolemia, hepatomegaly, pruritus, jaundice, hypertriglyceridemia, xanthomas, esophageal varices, and hepatocellular carcinoma (Kamath et al. 2018). It is noteworthy that patients with ALGS have a higher level of bilirubin and a higher pediatric end-stage liver disease score than age-matched patients with biliary atresia ($P<0.001$) (Kamath et al. 2012).

Pathophysiology of Cholestatic Pruritus

The pathophysiologic mechanism of cholestatic pruritus has not been identified. However, a pruritogen that accompanies BAs may be a culprit. Possible pruritogens are biliary agents, endogenous opioids, and the autotaxin-lysophosphatidic acid axis. A recently identified bile salt subspecies was found to induce pruritus via subreceptor X4 of the Mas-related G-protein coupled receptor family. When subreceptor X4 of the Mas-related G-protein coupled receptor family is injected intradermally in humans, itch is produced. This mechanism, however, does not completely account for the pruritus of cholestasis (Meixiong et al. 2019; Yu et al. 2019). A role for endogenous opioids in the mechanism of cholestatic pruritus has been proposed, however, there are no convincing data to show a correlation between levels of endogenous opioids (such as preproenkephalin mRNA) and intensity of itch (Bergasa et al. 1995). The phospholipid, lysophosphatidic acid, and its hydrolyzing enzyme, autotaxin, were found in higher concentrations in the sera of patients with cholestatic pruritus compared with patients without pruritus. Furthermore, a correlation exists between levels of autotaxin and the intensity of itch and levels of bile salts (Kremer et al. 2012; Keune et al. 2016; Kremer et al. 2016).

Diagnosis of Cholestatic Pruritus

Cholestasis pruritus is a clinical diagnosis. It is based on the presence of cholestasis and pruritus without skin lesions.

Treatment of Cholestatic Pruritus

Cholestyramine

Cholestyramine, an anion-exchange resin that binds BAs and blocks BA absorption in the terminal ileum, is the only approved therapy for cholestatic pruritus. Although cholestyramine may improve cholestatic pruritus after 2 weeks of treatment, it is often inadequate as monotherapy in achieving therapeutic benefit. In addition, it is associated with potential for GI adverse effects and can inhibit the absorption of other medications.

The following therapies are used in clinical care but have not been approved for treatment of cholestatic pruritus (Thebaut et al. 2017):

- Local care, such as topical emollients.
- Ursodeoxycholic acid (UDCA): UDCA is often used as first-line therapy, with some reports of improvement in pruritus and xanthomas in ALGS.

- Rifampicin, an enzyme inducer which may increase metabolism of potential pruritogens in the liver. Rifampicin is considered second- or third-line therapy due potential hepatic and hematologic complications.
- Opioid antagonists: such as naltrexone, are third- or fourth-line therapies for pruritus in patients with ALGS and are associated with some improvement of pruritus (Kronsten et al. 2013).
- Ondansetron: a 5-hydroxytryptamine antagonist, improves pruritus compared with placebo (Müller et al. 1998).
- Barbiturates: Phenobarbital is occasionally used as a rapid inducer of hepatic microsomal drug oxidizer, to decrease the concentration of pruritogens; however, it is associated with untoward sedating effects.
- Antihistamines: Cholestatic pruritus is not associated with a rise in histamine levels and nonsedating antihistamines are not efficacious. However, sedating antihistamines may be administered at night to assist with sleep.
- Combination therapy is used in a majority of patients because a single therapy typically does not provide adequate relief of pruritus. Many patients continue to suffer from intractable pruritus despite receipt of maximal medical therapy.
- Partial external biliary diversion and ileal exclusion: Partial external biliary diversion and ileal exclusion promote BA loss and have been effective for treating cholestatic pruritus in ALGS.
- Orthotopic liver transplantation: Liver transplantation for end-stage liver disease and intractable cholestatic pruritus is an established treatment for patients with ALGS.

Hence a therapeutic gap exists for pruritus in ALGS—the most common cause of disabling cholestatic pruritus in children.

3.1. Review Issue List

3.1.1. Key Review Issues Relevant to Evaluation of Benefit

3.1.1.1. Regulatory Framework for Establishing Substantial Evidence of Effectiveness

3.1.1.2. Randomized Study Withdrawal Design

3.1.1.3. Uncertainty of Itch Reported Outcome Instrument (ItchRO[Obs]) Item 1

3.1.1.4. No Single Prespecified Pruritus Endpoint

3.1.1.5. Evaluation of Clinically Meaningful Within-Patient Change in Pruritus

3.1.2. Key Review Issues Relevant to Evaluation of Risk

3.1.2.1. Liver Test Abnormalities

3.1.2.2. Diarrhea, Vomiting, Abdominal Pain

3.1.2.3. Fat-Soluble Vitamin Deficiency

3.1.2.4. Limited Placebo-Control Safety Database

3.2. Approach to the Review

Table 4 provides an overview of the clinical studies conducted to support the benefit-risk assessment of maralixibat. Results of the randomized withdrawal (RWD) period of LUM001-304 provide the primary efficacy data of maralixibat for treatment of pruritus in pediatric patients with ALGS. Study LUM001-304 (open-label extension [OLE] and RWD periods) provide the primary safety data.

In addition, supportive randomized placebo-controlled Studies LUM001-301 and LUM001-302 with their long-term extensions (LUM001-305 and LUM001-303, respectively), provide supportive evidence for safety.

Note regarding description(s) of dose(s) throughout the review. During drug development, the dose was described in terms of maralixibat chloride (the salt form). Therefore, 400 mcg/kg/day maralixibat chloride in the pivotal study is equivalent to 380 mcg/kg/day maralixibat (base form) in the label. This also applies to other dose descriptions throughout the review (e.g., 70 mcg is equivalent to 66.5 mcg, 140 mcg is equivalent to 133 mcg, and 280 mcg is equivalent to 266 mcg). Throughout the review, the dose may be described in the salt (maralixibat chloride) or base (maralixibat).

Table 4. Clinical Studies Submitted in Support of Efficacy and/or Safety Determinations[1] for Maralixibat

Identifier (NCT#)	Study Population	Study Design	Regimen (Number. Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized[2]	Number of Centers and Countries
LUM001-301 (NCT02057692)	Pediatric patients with ALGS	Multicenter, randomized, double-blind, placebo-controlled	70 mcg/kg: N=8 140 mcg/kg: N=11 280 mcg/kg: N=6 Placebo: N=12 Duration: 13 weeks	Mean change from baseline to Week 13 in pruritus as measured by ItchRO(Obs) weekly average score	Planned: N=36 Actual: N=37	13 Centers US Canada
LUM001-302 (NCT01903460)	Pediatric patients with ALGS	Multicenter, randomized, double-blind, placebo-controlled	140 mcg/kg: N=6 280 mcg/kg: N=8 Placebo: N=6	Mean Change from baseline to Week 13 in fasting sBA level	Planned: N=18 Actual: N=20	3 Centers UK
LUM001-303 (NCT02047318)	Pediatric patients with ALGS	Open-label, long-term, extension (of LUM001-302)	Drug: 280 mcg/kg: N=19 Option for 280 mcg/kg BID: N=5 of 19	Mean change from baseline to Week 48 in fasting sBA level	Actual: N=19	3 Centers UK
LUM001-304 (NCT02160782)	Pediatric patients with ALGS	Open-label run-in with 4-week double-blind, placebo-controlled randomized withdrawal (RWD) period	400 mcg/kg QD: OL Run-In: N=31 RWD:N=29 400 mcg/kg: N=13 Placebo: N=16 After RWD: N=29 LTE: N=23 LTE: 400 BID: N=14 of 23	Mean change from Week 18 to 22 of fasting sBA level in participants who had a reduction in sBA ≥50% from BL to Week 12 or 18	Planned: N=30 Actual: N=31	9 Centers Australia Belgium France Spain Poland UK
LUM001-305 (NCT02117713)	Pediatric patients with ALGS	Open-label, long-term, extension (of LUM001-301)	280 mcg/kg OL: N=34	Mean change from baseline to Week 48 in fasting sBA level	Actual: N=34	11 Centers US Canada

Source: Reviewer
[1] Includes all submitted clinical trials, even if not reviewed in-depth, except for Phase 1 and pharmacokinetic studies.
[2] If no randomization, then replace with "Actual Enrolled"
Abbreviations: ALGS, Alagille syndrome; BID, twice daily; BL, baseline; DB, double-blind; ItchRO(Obs), itch reported outcome (observer); LTE, long-term extension study; MC, multicenter; N, number of subjects; OL, open-label; PC, placebo-controlled; PG, parallel group; QD, once a day

4. Patient Experience Data

The Applicant submitted patient experience data (Table 5) to assess the efficacy of maralixibat. The itch reported outcome (observer) ItchRO(Obs) clinical outcome assessment measured patients' scratching as observed by their caregiver and scores were recorded in an eDiary in the morning and the evening. ItchRO(Obs) is a 5-point ordinal response scale, with scores ranging from 0 (no scratching) to 4 (worst possible scratching). Table 5 summarizes the ItchRO(Obs) data as discussed in this Integrated Review as well as the Applicant's submission of other patient experience data.

Table 5. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
Clinical outcome assessment data submitted in the application		
☒	Patient-reported outcome	Integrated Review: Section 6: Evidence of Benefit Section III.16: Additional Information and Assessment Submission: Module 2.5: Clinical Overview Module 2.7.3: Summary of Clinical Efficacy Module 5.3.5.1: Study Reports of LUM001-301, -302, -304 Module 5.3.5.2: Study Reports of LUM001-303 and-305
☒	Observer-reported outcome	Integrated Review: Section 6: Evidence of Benefit Section III.16: Additional Information and Assessment Submission: Module 2.5: Clinical Overview Module 2.7.3: Summary of Clinical Efficacy Module 5.3.5.1: Study Reports of LUM001-301, -302, -304 Module 5.3.5.2: Study Reports of LUM001-303 and-305

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
☒	Clinician-reported outcome	Integrated Review: Section III.16: Additional Information and Assessment Submission: Module 2.5: Clinical Overview Module 2.7.3: Summary of Clinical Efficacy Module 5.3.5.1: Study Reports of LUM001-301, -302, -304 Module 5.3.5.2: Study Reports of LUM001-303 and-305
☐	Performance outcome	
Other patient experience data submitted in the application		
☐	Patient-focused drug development meeting summary	
☒	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	Submission: Module 5.3.5.4: Development of the Pediatric ItchRO
☐	Observational survey studies	
☒	Natural history studies	Submission: Module 2.7.4: Summary of Clinical Safety
☐	Patient preference studies	
☐	Other: (please specify)	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

Table 6. Summary of General Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information
Pharmacologic Activity	
Established pharmacologic class	ileal bile acid transporter (IBAT) inhibitor
Mechanism of action	Maralixibat is a reversible inhibitor of the IBAT. It decreases the reabsorption of bile acids (primarily the salt forms) from the terminal ileum. Pruritus is a common symptom in patients with ALGS and the pathophysiology of pruritus in patients with ALGS is not completely understood. Although the mechanism by which maralixibat improves pruritus in Patients with ALGS is unknown, it may involve inhibition of the IBAT, which results in decreased reuptake of bile salts, as evidenced by a decrease in serum bile acids
Active moieties	Maralixibat
QT prolongation	Maralixibat is not expected to prolong the QT interval. Based on low systemic exposure to maralixibat (~4nM) in the food effect study that involved doses higher (100 mg) than the maximum recommended therapeutic dose (up to 26.5 mg), a thorough QT study was not deemed necessary per the IRT-QT (see the IRT-QT review memo in DARRTS by Dr. Nan Zheng dated 04/19/2021).
General Information	
Bioanalysis	Plasma concentrations of maralixibat were measured using adequately validated assay using an LC-MS/MS based method.
Healthy subjects versus patients	N/A due to the low systemic exposure of maralixibat
Drug exposure at steady state following the therapeutic dosing regimen (or single dosage, if more relevant for the drug)	Concentrations of maralixibat in pediatric patients with ALGS were below the limit of quantification (0.25 ng/mL) in the majority of plasma samples. In Study LUM001-304, the highest concentration of maralixibat in pediatric patients with ALGS following treatment with maralixibat 400 µg/kg (380 µg/kg/day free-base equivalent) once daily was 5.93 ng/mL. Following single oral administration of maralixibat in healthy adults at doses ranging from 1 mg to 500 mg, plasma concentrations of maralixibat were below the limit of quantification (0.25 ng/mL) at doses less than 20 mg and PK parameters could not be reliably estimated. Following a single-dose administration of 30 mg under fasted conditions, median T_{max} was 0.75 and mean (SD) C_{max} and AUC_{last} were 1.65 (1.10) ng/mL and 3.43 (2.13) ng·h/mL, respectively.
Range of effective dosage(s) or exposure	The Applicant's proposed target dose 400 µg/kg QD (380 µg/kg/day free-base equivalent) was evaluated in the placebo-controlled pivotal Study LUM001-304. In phase 2 trials, doses lower than 400 µg/kg/day were studied but did not show significant effects on pruritus or sBA compared to placebo. Doses higher than 400 µg/kg QD were not formally evaluated for efficacy. Although dose escalation to a higher dose of maralixibat 800 µg/kg/day (400 µg/kg twice daily as maralixibat salt) was allowed in the open-label long term extension, the dose-response relationship for efficacy at doses higher than 400 mcg/kg could not be evaluated due to the limitations in the study design. (see Section III.14.2.5).

Characteristic	Drug Information
Maximally tolerated dosage or exposure	In the pivotal trial (LUM001-304), doses up to 400 µg/kg/day (380 µg/kg/day free-base equivalent) were evaluated during the randomized withdrawal phase. During the long-term treatment period (long-term extension phase [LTE]), the dose of maralixibat was increased to a maximum of 800 µg/kg/day (400 µg/kg twice daily) in pediatric patients with ALGS. During the long-term extension period, there were drug discontinuations due to elevated ALT and TB including at least one event that was adjudicated as probable (with some as possible) association with drug and two patients had to reduce the dose from 800 µg/kg/day for elevated ALT. However, it was difficult to separate disease progression from treatment-emergent AEs given these were in open-label studies.
Dosage proportionality	Following a single oral administration of maralixibat at doses of 30, 45, and 100 mg under fasted conditions in healthy subjects, AUC_{last} increased in a dose-related manner with an increase of 4.6-fold for a 3.3-fold dose increase from 30 to 100 mg; C_{max} increased 2.4-fold.
Accumulation	Accumulation of maralixibat was not observed following repeated oral administration in healthy adults at doses up to 100 mg once daily.
Bridge between to-be-marketed and clinical trial formulations	The to-be-marketed formulation is different from those evaluated in the clinical studies. In clinical trials, the body weight-based dose was provided using oral solution as multiple concentrations and a fixed dosing volume (i.e., 1 mL for patients <10 kg and 5 mL for patients >10 kg). By contrast, the to-be-marketed formulation is a single-strength oral solution. Body weight-based dosing will be provided by adjusting dosing volumes. Maralixibat oral solution FDV (formulation used in clinical studies) and FDSC (to-be-marketed formulation) have minor changes in their composition, which are believed not to alter efficacy and safety of the formulation. The drug product is intended to target small-intestine surface with minimal absorption systemically. Further, the oral solutions do not contain excipients that can significantly affect drug absorption. Therefore, a bridging BE study between the formulation used in the clinical studies and the TBM formulations was not deemed necessary. Refer to the biopharmaceutics review for additional comments.
Absorption	
Bioavailability	The absolute bioavailability of maralixibat has not been characterized. The mass balance study results indicate the oral absorption is low (<1%).
T_{max}	0.5-7.5 h
Food effect (fed/fasted); geometric least square mean and 90% CI	Concomitant administration of a high-fat meal with a single oral dose of maralixibat decreased both the rate and extent of absorption. AUC and C_{max} of maralixibat in the fed state were 64.8% to 85.8% lower relative to oral administration of 30 mg in fasted conditions. The effect of food on changes in systemic exposure to maralixibat is not expected to be clinically significant.
Distribution	
Plasma protein binding	The specific protein binding of maralixibat in human serum albumin was >91% and concentration independent over the 0.025-25 µg/mL range. The specific protein binding of maralixibat in alpha-1-acid glycoprotein was >93% and independent of concentration over the range of concentrations evaluated. The mean percentages of plasma protein binding were 95.9, 97.3 and 96% at concentrations of 0.25, 2.5, and 25 µg/mL, respectively.
Drug as substrate of transporters	The interaction of maralixibat with human MDR1, BCRP, OCT3, OATP1B1, OATP2B1, OCTN1, OCTN2, MRP2, and PEPT1-transporters was assessed at concentrations up to 10µM. Maralixibat is not a substrate of BCRP, MDR1, OATP1B1, OATP1B3, and OATP2B1 transporters.
Elimination	

Characteristic	Drug Information
Mass balance results	Following a single oral dose of radiolabeled 5 mg maralixibat, less than 1% of the radioactive dose was detected in plasma, whole blood, or urine. Total radioactivity recovery was 72.532%, with 72.466% found in the feces and 0.066% in the urine. More than 94% of fecal radioactivity was determined to be unchanged maralixibat.
Half-life	Following a single oral dose of 30 mg maralixibat in healthy adults, the mean half-life (t1/2) was 1.6 h.
Metabolic pathway(s)	In in vitro studies, metabolism of maralixibat occurred via two major routes: N-demethylation to form M1 and hydroxylation to form M3. The subsequent hydroxylation of M1 or N-demethylation of M3 yielded M4. However, no metabolites were detected in plasma in vivo, and <3% of the total radioactivity was associated with the three metabolites detected in feces (M1, M3, and M4).
Primary excretion pathways (% dosage)	Fecal excretion (see 'Mass balance results' above)
Intrinsic Factors and Specific Populations	
Renal impairment	No dedicated renal impairment study was conducted. Given the low systemic absorption, significant effects of renal impairment on PK are unlikely. In clinical trials, patients with renal impairment were not included
Hepatic impairment	No dedicated hepatic impairment study was conducted. In clinical trials, patients with ALGS have abnormal liver laboratory values including elevated total bilirubin level at baseline. Patients with cirrhosis were not enrolled in the clinical trials.
Drug Interaction Liability (Drug as Perpetrator)	
Inhibition/induction of metabolism	Maralixibat inhibits CYP3A4 in vitro; however, clinically relevant effects on the pharmacokinetics of CYP3A4 substrates are expected to be minimal.
Inhibition/induction of transporter systems	Maralixibat inhibits the transporter OATP2B1 in vitro, which can potentially result in reduced absorption of drugs that rely on OATP2B1-mediated uptake in the gastrointestinal tract. In clinical studies, coadministration of 4.75 mg maralixibat (68 µg/kg for a 70 kg patient; administered before breakfast) with daily doses of simvastatin or lovastatin administered in the afternoon per their labeling, or atorvastatin administered in the morning or in the afternoon per its labeling did not have a clinically relevant effect on the pharmacokinetics of these statins and their metabolites. However, the effect of maralixibat on the pharmacokinetics of OATP2B1 substrates at the higher doses has not been evaluated in a clinical study.

Source: Reviewer generated table based on Applicant's data
Abbreviations: ALGS, Alagille syndrome; $AUC_{last,}$ area under the curve to the last measured concentration; AUC area under the curve; BRCP, breast cancer resistance protein; CYP, cytochrome P450; BE, bioequivalence; IBAT, inhibitor of the ileal bile acid transporter; C_{max}, maximum plasma concentration; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MRP, multidrug resistance associated protein; OATP, organic anion transporting polypeptide; OCTN, organic cation transporter; PEPT, peptide transporter; P-gp, P-glycoprotein; PK, pharmacokinetic; QD, once a day; T_{max}, time to maximum plasma concentration; TBM, to-be-marketed

5.1. Nonclinical Assessment of Potential Effectiveness

Livmarli (maralixibat chloride) is an inhibitor of the apical sodium-dependent bile acid transporter (ASBT) (also known as the ileal bile acid transporter [IBAT]). The ASBT is a transmembrane protein localized on the luminal surface of ileal enterocytes. Maralixibat was shown to inhibit bile acid reabsorption using in vitro and in vivo methods (nonclinical studies previously reviewed in INDs (b) (4) and 119917). Details of the nonclinical pharmacology studies are provided in Sections III.13.1.1 and III.13.2.1. ALGS is a cholestatic liver disease for which no animal model has been established. Therefore, the Applicant evaluated the potential effectiveness of maralixibat in a rat cholestasis model that involved partial bile duct ligation (pBDL), to simulate the reduced bile flow in Patients with ALGS and other cholestatic liver diseases. pBDL leads to increases in serum bile acid (sBA) levels and several parameters of liver toxicity and function (e.g., alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma-glutamyl transferase [GGT], alkaline phosphatase [ALP], and bilirubin).

In the current NDA, the nonclinical assessments of maralixibat for its effects on serum bile acid levels, fecal excretion of BAs, and liver injury enzyme markers in a rat pBDL cholestasis model are reviewed. Maralixibat was tested as a monotherapy or in combination with ursodeoxycholic acid (UDCA), a drug approved for the treatment of primary biliary cirrhosis.

The major findings from the in vivo primary pharmacodynamic studies in rats, dogs, and monkeys are summarized below.

- Once-daily oral dosing of up to 2 mg/kg maralixibat for 4 days in Wistar rats or 150 mg/kg/day for 14 days in Sprague-Dawley rats resulted in a dose-dependent, significant increase in fecal BA excretion, ranging from 1.2- to 11.1-fold compared to the controls.
- In overnight fasted dogs, SD-5613 (maralixibat) produced a dose-dependent inhibition in the post-prandial rise in serum total BAs, with 50% effective dose of 0.2 mg/kg.
- In dogs, maralixibat at oral doses of 1, 2, and 4 mg/kg/day (in solution) or 2 mg/kg/day in capsules produced a dose-dependent increase in fecal total BAs after 1 or 2 weeks of treatment.
- The treatment of cynomolgus monkeys for 7 days with maralixibat by nasogastric gavage (0.1 to 20 mg/kg/day at 30 min prior to feeding), or rhesus monkeys for 2 weeks with 5 or 50 mg/kg/day (gelatin capsules) resulted in dose-dependent increases in fecal BA when compared to the control values.
- The effects of maralixibat on total serum cholesterol were variable among species. Rats showed an increase in total cholesterol at doses of 5 to 150 mg/kg/day given for 4 weeks. In dogs, reductions in total cholesterol and high-density lipoprotein cholesterol were observed at oral doses of 1 and 4 mg/kg/day administered for 14 days.
- In the rat partial bile duct ligation (pBDL) model of cholestasis, maralixibat alone at oral doses of 0.3, 1, and 10 mg/kg/day or the combination of maralixibat (1 mg/kg/day) and UDCA (1 mg/kg/day) reduced serum bile acids (sBA), ALP, AST, ALT, GGT, and total bilirubin after 14 days of treatment. The combined treatment showed a slightly greater improvement in these biomarkers as compared to maralixibat or UDCA alone.

Maralixibat is the second IBAT inhibitor submitted for marketing approval in the United States, and one drug with the same mechanism of action has been approved with the following established pharmacologic class (i.e., Established Pharmacologic Class text phrase): “ileal bile acid transporter (IBAT) inhibitor.” Therefore, the same Established Pharmacologic Class text phrase should be used for maralixibat.

6. Assessment of Effectiveness

6.1. Dose and Dose Responsiveness

6.1.1. Is the Proposed Dose of 400 mcg/kg (380 mcg/kg Free-Base Equivalent) Once Daily Reasonable?

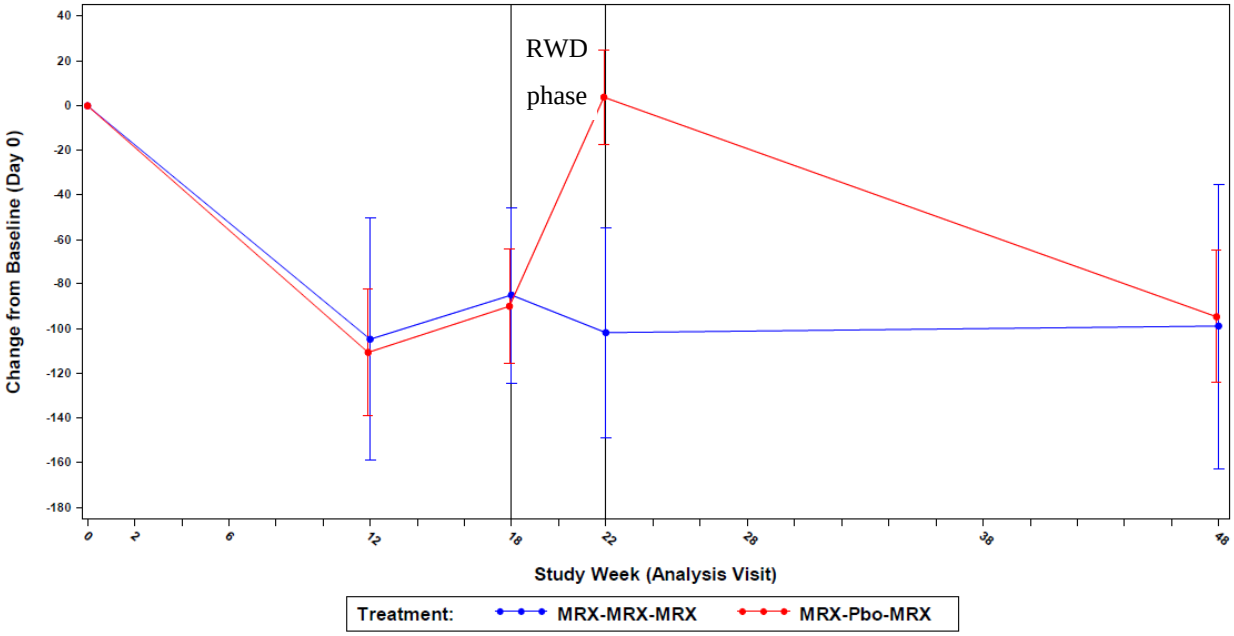
The Applicant’s proposed dose of 400 mcg/kg QD (380 mcg/kg maralixibat free base) was evaluated in the placebo-controlled randomized withdrawal study (LUM001-304).

Doses lower than 400 mcg/kg/day (66.5, 133, 266 mcg/kg) were studied in two placebo-controlled phase 2 trials (LUM001-301 and LUM00-302) but did not show significant improvement on pruritus score or decrease in sBA compared to placebo. Because no significant effects on sBA and pruritus were shown at lower doses in phase 2 trials, 400 mcg/kg was studied in LUM001-304. Refer to Section III.14.2 for more details.

The pivotal Study LUM001-304 was a randomized, placebo-controlled study with a drug-withdrawal period in pediatric patients with ALGS ≥12 months of age. Refer to Section 6.2.1 for details of the study design. In Study LUM001-304, patients who were treated with placebo during the 4-week randomized-withdrawal (RW) period from Week 18 to Week 22 had a worsening of itching, as measured by ItchRO score, whereas those who remained on maralixibat 380 mcg/kg maintained a low ItchRO score on average, leading to differences in mean ItchRO scores between the maralixibat and placebo groups (Figure 5). Refer to Section 6.2.1 for more comments.

Upon maralixibat treatment, sBA levels were lowered and remained lower over time (Figure 1). In the RWD period, an increase in sBA levels in the placebo group was observed while the lowered sBA levels was maintained in the maralixibat-treated patients (see Section 6.2.1 for additional analysis). The increase in sBA in the placebo group during the RWD was trending with the increase in ItchRO score. We note that the bioanalytical method for serum bile acid levels was not adequately validated according to the FDA guidance for industry Bioanalytical Method Validation (2018). As a result, the reliability of the reported serum bile acid values cannot be assured and therefore, the results of serum bile acid levels only allow for assessment of overall trends in a qualitative manner (see Section III.14.5 for additional details pertaining to the measurement of sBA).

Figure 1. Plot of Mean (± SE) Change from Baseline in sBA (µmol/L) by Randomized Treatment Group Over Time (through Week 48) Trial LUM001-304



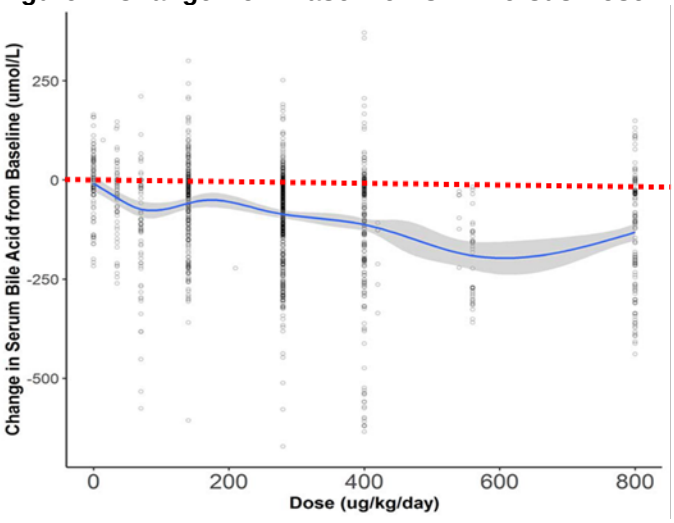
Source: Figure 14.2.17.1.1 from CSR for Trial LUM001-304.
Abbreviation: sBA, serum bile acids

The Applicant also evaluated lower doses during the clinical development program. In supportive Study LUM001-301, a randomized, doubled-blinded, placebo-controlled, parallel group study in patients with ALGS 1 to 17 years of age (n=37), patients were randomized to doses of 70 to 280 mcg/kg/day (66.5 to 266 mcg/kg/day free-base equivalent) with an 8-week stable-dose treatment period after a 5-week initial dose-escalation period. In this study, no statistically significant improvement on sBA or pruritus in the maralixibat treatment arms was observed compared to placebo. In addition, there was no dose-response in the sBA and pruritus endpoints (see Section III.14.2 for details).

Doses higher than 400 mcg/kg/day (380 mcg/kg/day free base equivalent) were not studied in controlled trials. A dose increase to 400 mcg/kg/day (380 mcg/kg/day free base equivalent) twice daily was allowed in the optional, open-label long-term extension (LTE) period in Study LUM001-304. Only patients who choose stayed in the optional LTE period after a 24-week long-term stable dosing period at 400 mcg/kg/day and may have increased their dose based on sBA levels and ItchRO[Obs] score and safety. Due to the limitations of the study design, a dose-response relationship for efficacy at doses higher than 400 mcg/kg/day (380 mcg/kg/day free base equivalent) cannot be adequately evaluated.

When sBA data were pooled across the studies (LUM001-301, LUM001-302, LUM001-303, LUM001-304, and LUM001-305) for a dose-response analysis, there was no apparent dose-response relationship in sBA change across the doses assessed in the clinical program (Figure 2).

Figure 2. Change from Baseline—sBA Versus Dose



Source: Exploratory Dose-Response Analysis of Maralixibat and Serum Bile Acid, Figure 2.
Abbreviation: sBA, serum bile acids

6.1.2. Is the Proposed Dose-Escalation Strategy Starting From 200 mcg/kg/Day (190 mcg/kg/Day Free-Base Equivalent) for 1 Week to a Target Dose of 400 mcg/kg/Day (380 mcg/kg/Day Free-Base Equivalent) Acceptable?

The proposed starting dose of 200 mcg/kg/day (190 mcg/kg/day free-base equivalent) and dose escalation strategy in one step to a target dose of 400 mcg/kg/day (380 mcg/kg/day free-base equivalent) is acceptable. Although this dosing regimen has not been evaluated in the pivotal trial LUM001-304, the Applicant has collected safety data using the proposed dose escalation scheme in their expanded access program for ALGS patients.

In the pivotal Study LUM001-304 in pediatric patients with ALGS, dose was escalated gradually from 14 mcg/kg/day to a target dose of 400 mcg/kg/day over the 6-week dose escalation period. In the Phase 2 studies LUM001-301 and LUM001-302 in pediatric patients with ALGS, doses were also escalated gradually from 14 µg/kg/day to the target dose over 3 to 5 weeks of the dose-escalation period. Furthermore, in the extension Studies LUM001-305 and LUM001-303 in pediatric patients with ALGS, doses were escalated from 14 mcg/kg/day to the target dose over 3 to 5 weeks in patients initially receiving placebo in Studies LUM001-301 and LUM001-302, respectively. Refer to Section 6.2 for details of the clinical trial design for Study LUM001-304. In the long-term open-label extension period of pivotal Study LUM001-304, in patients who experience ≥7 days of interruption in maralixibat dosing, maralixibat was restarted at 35 mcg/kg/day and escalated to the target dose of 400 mcg/kg/day over the 4 weeks of the dose-escalation period.

In the extension phase of LUM001-304, the afternoon dose was added starting with dose of 140 mcg/kg/day for 4 weeks then escalated to 400 mcg/kg/day, up to 400 mcg/kg BID (a total daily dose of 800 mg/kg/day,760 mcg/kg/day free base equivalent). However, these ALGS patients in extension phase of LUM001-304 were not treatment naive ALG patients and had already been treated with 400 mcg/kg/day for an extended period. As such the proposed rapid dose escalation scheme was not studied in the clinical trials.

The Applicant has indicated that the proposed dosing regimen is supported by the lack of safety findings in the extension phase of LUM001-304, where the afternoon dose was increased in only one step from 140 mcg/kg for 4 weeks to 400 mcg/kg, up to a total daily dose of 800 mcg/kg (760 mcg/kg free-base equivalent). Further, all participants were escalated to 400 mcg/kg once daily and no dose-limiting or dose-dependent safety findings were observed.

The Applicant further indicated that maralixibat has been administered without dose escalation in healthy subjects in the single ascending dose study (14 to 7000 mcg/kg; Study NB4-02-06-002) and multiple ascending doses study (7 to 1400 mcg/kg; Study NB4-02-06-003), in obese adults (10 to 100 mg QD or 50 mg BID; 140 to 1400 mg/kg QD or 700 mg/kg BID equivalent; Study SHP625-101), and in adult patients with hypercholesterolemia (1 to 40 mg QD and 1 to 5 mg BID; 14 to 560 mcg/kg QD, and 14 to 70 mcg/kg BID; Study NB4-03-06-009) without significant safety findings. However, there are limitations in extrapolating safety data from healthy adults or adults with other indications (obese, or with hypercholesterolemia) to the pediatric patients with ALGS.

As a part of the 120-day safety update, the Applicant shared the safety data from their expanded access program (EAP) for patients with ALGS. Participants in the EAP were dosed with a 200 mcg/kg dose of maralixibat chloride once daily for the first 7 days, followed by 400 mcg/kg once daily in the absence of major safety or tolerability findings. The safety population in this program comprised 24 patients, 22 of whom were treatment naïve. Of the 24 participants in the EAP who received maralixibat, 2 experienced treatment-emergent adverse events (TEAEs) (Grade 1 emesis and Grade 1 diarrhea; 1 participant each). One participant (4-year-old male) was reported to take an overdose (300 μg/kg twice daily) for approximately 1 month instead of the correct dosage of 200 mcg/kg once daily followed by 400 mcg/kg once daily after 1 week. There were no adverse events (AEs) associated with the overdose, and no treatments or procedures were required. In response to an Information Request (IR) dated May 26, 2021, the Applicant provided additional demographics and baseline laboratory values for the participants in the EAP (Table 7), which indicated that the characteristics of the patients in the EAP are consistent with the proposed indication.

While we note that the dose-escalation scheme evaluated in the pivotal trial (LUM001-304) is not consistent with the proposed dosing scheme, the safety data from the EAP supports the safety of the proposed starting dose of 200 mcg/kg once daily for 1 week followed by dose escalation to 400 mcg/kg in treatment-naïve pediatric patients with ALGS.

Table 7. Expanded Access Program Baseline Patient Demographics

Variable Statistics or Category	Expanded Access Program Patient (N = 36)[1]
Age (years) [2]	
n	35
Mean	7.1
SD (SE)	5.44 (0.92)
Median	6.0
Minimum, Maximum	1, 27
Sex	
Male	19 (52.8%)
Female	17 (47.2%)
Baseline Height (cm) [3]	
N	14
Mean	102.1
SD (SE)	23.34 (6.24)
Median	105.0
Minimum, Maximum	70, 154
Baseline Weight (kg) [3]	
n	14
Mean	18.7
SD (SE)	13.39 (3.58)
Median	15.5
Minimum, Maximum	9, 60

1 All available demographic information from the 36 participants who have enrolled in the EAP is included. 24 participants have a reported first dose date of MRX.
2 Only birth year was collected so the age at baseline was derived assuming a birthday of 01-Jan.
3 Height and weight were only collected for participants who provided consent for additional data collection.

Source: 1.11.3 Clinical Information Amendment Submitted on June 2, 2021
Abbreviations: SD, standard deviation; SE, standard error; MRX, maralixibat

6.2. Clinical Trials Intended to Demonstrate Efficacy

6.2.1. Trial LUM001-304

Long-Term, Open-Label Study with a Double-Blind, Placebo-Controlled, Randomized Drug Withdrawal Period of LUM001, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi), in Patients with Alagille Syndrome.

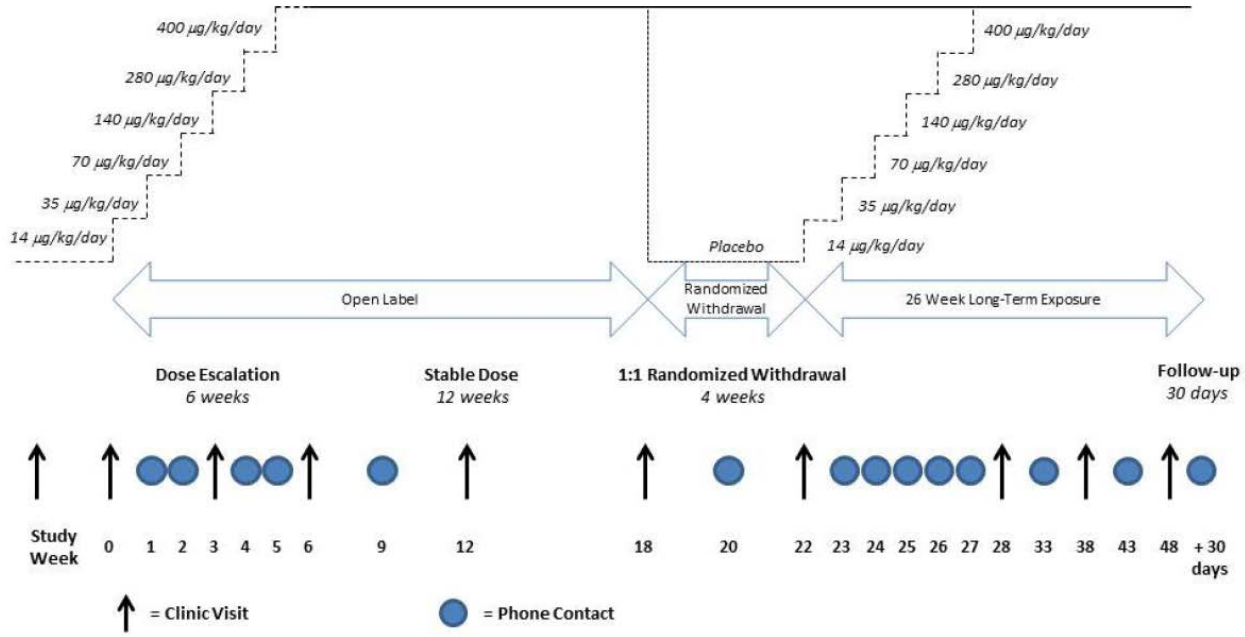
6.2.1.1. Design, Study LUM001-304

Study LUM001-304 (NCT02160782) was designed as a Phase 2 study entitled “ICONIC: Long-Term, Open-Label Study with a Double-Blind, Placebo-Controlled, Randomized Drug

Withdrawal Period of LUM001, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi), in Patients with Alagille Syndrome."

The original LUM001-304 protocol planned for a 48-week study period, and several protocol amendments allowed optional long-term treatment beyond Week 48. The study consisted of an 18-week open-label (OL) run-in period; a 4-week randomized, double-blind, placebo-controlled RWD period; a subsequent 26-week OL treatment period (after randomized withdrawal [ARW] phase); and an optional LTE treatment period. A schema of the initially planned 48 weeks of the study, excluding the LTE period, is depicted in Figure 3.

Figure 3. Study LUM001-304 Schema



Source: Applicant's Figure 3-1 on page 33 of the Clinical Study Report for LUM001-304.

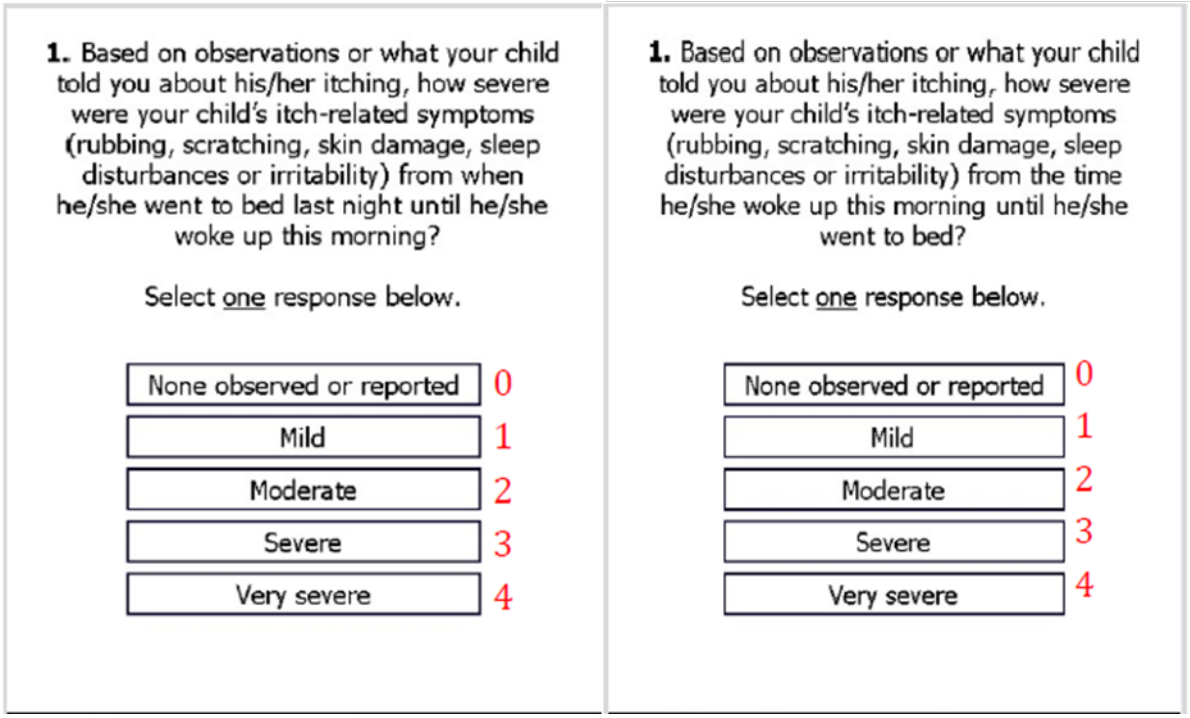
After OL treatment through Week 18, all patients remaining in the study were randomized in a 1:1 ratio to receive maralixibat or placebo in the 4-week RWD period. Note that this randomization of all patients remaining in the study at Week 18 differs from certain designs for maintenance studies where only responders are randomized in the RWD period. Randomization was stratified by whether the patient achieved a >50% reduction in sBA between baseline and Week 12 according to the protocol versions up to and including Amendment 3 (dated November 13, 2015). In a response dated May 20, 2019 under IND 119917, the Applicant stated that all patients were randomized prior to protocol Amendment 4 (dated March 28, 2017), which specifies the stratification criterion for randomization slightly differently (refer to Section III.16.2 for the dates of all protocol amendments). The randomization lists and ordering submitted by the Applicant demonstrate that randomization was also stratified by study site.

Following the 4-week RWD period, patients who had received placebo subsequently received maralixibat according to a dose-escalation schedule similar to the initial escalation. Subjects who were randomized to receive maralixibat underwent a simulated dose escalation to maintain the blinding. Dosing with maralixibat continued in the long-term treatment periods.

Due to the youth of the patients, the primary assessment of pruritus was based on an observer-reported assessment of the patient's scratching. Caregivers assessed patients' itch-related

symptoms twice daily (once in the morning and once in the evening) on item 1 of the ItchRO(Obs) using the 5-point ordinal response scale depicted in Figure 3. Scores range from 0 (none observed or reported) to 4 (very severe), with higher scores representing worse outcomes. Patients were included in Study LUM001-304 if the average scratching score in the 2 weeks prior to baseline was greater than 2 (moderate).

Figure 4. Observer-Reported Itch Outcome Instrument, ItchRO(Obs), Item 1, Morning and Evening



Source: Applicant's figure on pages 137 and 139 of the protocol dated February 8, 2019.
Left, morning report; right, evening report
Abbreviation: ItchRO(Obs), Itch Reported Outcome (Observer)

The Applicant also administered a patient-reported outcome, the itch reported outcome (patient) ItchRO(Pt), which was completed by patients who were old enough and able to self-report their itching (refer to Figure 40 and Figure 41). The protocol states that patients 9 years of age or older completed the ItchRO(Pt) independently, and patients 5 to 8 years of age, or where the investigator has expressed concern about the patient's ability to reliably complete the assessment (e.g., due to developmental delay), completed the ItchRO(Pt) with the help of a caregiver. Patients less than 5 years of age did not assess the ItchRO(Pt).

The primary endpoint specified in the protocol was the mean change from Week 18 to 22 in fasting sBA levels in subjects who previously responded to LUM001 treatment, as defined by a reduction in sBA of ≥50% from baseline to Week 12 or 18. Several secondary endpoints, including those related to pruritus, were not uniquely defined, and there was no planned adjustment for testing multiple endpoints.

The protocol states that *the secondary efficacy evaluations will include* the following:

- Change from Week 18 to Week 22 in:
  - Liver enzymes (ALT, ALP) and bilirubin (total and direct)
  - Pruritus as measured by ItchRO (Observer ItchRO/Patient ItchRO). Starting with protocol Amendment 4 (dated March 28, 2017), this was further specified to be

evaluated in subjects who previously responded to LUM001 treatment, as defined by a reduction in ItchRO scale of >1 point from baseline to Week 12 or Week 18

- Change from baseline to Week 18 in:
  - Fasting sBA levels
  - Liver enzymes (ALT, ALP) and bilirubin (total and direct)
  - Pruritus as measured by ItchRO (ItchRO[Obs]/ItchRO[Pt])

The protocol defined a daily ItchRO score as the higher of the ItchRO scores from the morning and evening, representing the most severe, or worst, itch over the 24-hour period. Baseline was defined as the average of the daily ItchRO scores in the 7 days immediately prior to Day 0. The weekly average of the daily scores was calculated for the defined study week, consisting of the 7 days prior to the study visit. The protocol also specified an additional evaluation of the daily scores using the average, rather than the higher, of the morning and evening scores.

To establish efficacy, maralixibat needs to demonstrate improvement in pruritus using an appropriate comparator. Therefore, pruritus results focused on the double-blind, placebo-controlled RWD period in patients continuously treated with maralixibat compared to patients treated with maralixibat for 18 weeks and subsequently withdrawn from maralixibat for 4 weeks are presented subsequently in this review.

The FDA's main analyses will be based on the endpoints evaluating weekly averages of the worst daily scores, because these summary measures were defined in the protocol. Due to the lack of prespecification of a single primary pruritus endpoint, the Agency investigated the endpoints listed below, resulting in valid, randomized treatment comparisons (Section 6.3.2):

- Weekly average worst daily ItchRO(Obs) scores at Week 22
- Change from Week 18 (pre-randomized treatment) to Week 22 in the weekly average:
  - Worst daily ItchRO(Obs) scores
  - Daily average of the morning and evening ItchRO(Obs) scores
  - Morning ItchRO(Obs) scores
  - Evening ItchRO(Obs) scores
- Change from baseline (pretreatment) to Week 22 in the weekly average:
  - Worst daily ItchRO(Obs) scores
  - Daily average of the morning and evening ItchRO(Obs) scores
  - Morning ItchRO(Obs) scores
  - Evening ItchRO(Obs) scores

The FDA also presents supportive results based on the patient-reported outcome, ItchRO(Pt), in patients of sufficient age and able to self-report their itching.

6.2.1.2. Eligibility Criteria, Study LUM001-304

Key Inclusion Criteria

- Pediatric patients ≥12 months to <18 years of age; with a diagnosis of ALGS based on diagnostic criteria (Table 8).
- Evidence of cholestasis (one or more of the following):
  - Total sBA >3× the upper limit of normal (ULN) for age.
  - Conjugated bilirubin >1 mg/dL.
  - Fat-soluble vitamin deficiency otherwise unexplainable.
  - GGT >3× the ULN for age.
  - Intractable pruritus explainable only by liver disease.
- Significant pruritus, defined as an average daily score of >2 on the ItchRO questionnaire for two consecutive weeks in the screening period.

Table 8. Alagille Syndrome Diagnostic Criteria

ALGS Family History Paucity	JAGGED1 or NOTCH2 Mutation	# Major Clinical Criteria Needed for Diagnosis[d]
Present or absent		
Present	Identified[b]	Any or no features
None (proband)		
Absent or unknown	Identified	1 or more features
Present	Not identified[c]	3 or more features
Absent or unknown	Not identified	4 or more features
Present		
Absent or unknown	Identified	Any or no features
Present	Not identified	1 or more features
Absent or unknown	Not identified	2 or more features

Source: Appendix 16.3 LUM001-304 Protocol

[a] Family history: ALGS in a first-degree relative.
[b] Identified: JAGGED1 or NOTCH2 mutation identified by a clinical laboratory.
[c] Not identified: Not identified on screening, or not screened for.
[d] Major clinical criteria for ALGS: cholestasis; consistent cardiac, renal, vascular, ocular, or skeletal involvement; or characteristic Alagille facies.
Abbreviation: ALGS, Alagille syndrome

Key Exclusion Criteria

- Chronic diarrhea requiring ongoing intravenous fluid or nutritional intervention
- Surgical interruption of the enterohepatic circulation or disease or condition that may interfere with bile-salt metabolism (e.g., inflammatory bowel disease)
- Liver transplant
- Decompensated cirrhosis
  - Presence of ascites, variceal hemorrhage, encephalopathy
  - International normalized ratio (INR) >1.5, ALT >15× ULN, albumin <3.0 g/dL
- Bile acid or lipid-binding resins within 28 days of screening
- History or presence of concomitant liver disease
- History of gallstones or kidney stones
- Pregnancy or lactation

- HIV or cancer (except for in situ carcinoma and cancers treated at least 5 years prior to screening with no evidence of recurrence)

6.2.1.3. Statistical Analysis Plan, Trial LUM001-304

The protocol and statistical analysis plans (SAPs) did not prespecify a single primary pruritus endpoint and did not specify a method to protect the study-wise type I error rate.

Protocol-Specified Analyses

All versions of the protocol state that secondary continuous measures, which would include pruritus outcomes, would be analyzed similarly to the primary analysis for the sBA primary endpoint. The primary endpoint was specified to be evaluated using an analysis of covariance (ANCOVA) model with treatment group as a factor and the Week-18 value as a covariate. The FDA additionally conducted an analysis of pruritus using an ANCOVA model with treatment group as a factor and the baseline (pretreatment) value as a covariate.

The protocol states that no multiplicity adjustments were made, and p-values from the secondary and exploratory efficacy analyses, including analyses of pruritus, were considered nominal.

When evaluating the worst daily score, the protocol states that if either the morning or evening report is not completed within the allowed reporting window, whichever report has been completed represents the daily score. If a patient or caregiver failed to complete both the morning and evening reports, the daily score for that day is treated as missing data.

A weekly average of daily scores is computed if at least four of the seven daily ItchRO scores for a 7-day period are reported. If fewer than four ItchRO scores are reported, then the weekly average from the previous compliant week will be used, i.e., the last observation carried forward method. The protocol states that missing data will not be imputed for other efficacy endpoints.

Statistical Analysis Plans

In the NDA submission, the Applicant provided the following milestone dates (additional milestones are described in Section III.16.2):

- **April 2016** – Last patient had their Week-22 study visit (the end of the RWD period)
- **October 2016** – Last patient had their Week-48 study visit
- **February 2018** – Statistical Interim Analysis Plan (SIAP) finalized
- **March 2018** – Database freeze, study team unblinded
- **March 2019** – First submission of SIAP and unblinded study results to the FDA
- **March 2020** – SAP finalized (incorporating data from the LTE period)

The SIAP dated February 4, 2018 covers analyses of data through Week 48 of the study but does not specify any formal statistical tests or analysis models for continuous endpoints. The SIAP specifies the same method for handling missing pruritus assessments as specified in the protocol.

Because the SAP dated March 17, 2020 was created after the study was unblinded and study results were available, the FDA review will focus on the prespecified analyses in the protocol and on sensitivity analyses of daily ItchRO summaries and methods for handling treatment interruption.

Handling Treatment Discontinuation/Interruption

The Applicant states that Patient LUM001-304-(b) (6) "was hospitalized during the randomized withdrawal period for a serious adverse event (SAE) of polytraumatism/splenic rupture which made it impossible for the participant to comply with in-clinic visits." The SAP dated March 17, 2020 (after unblinding of the study data) states that the primary analysis uses the "Week 22" clinic visit date "as the anchor in deriving Week 22 ItchRO weekly average scores." The SAP specifies a sensitivity analysis using the 4-week time period immediately after the Week-18 visit date to derive ItchRO weekly scores for Weeks 19, 20, 21, and 22.

According to the Applicant's response dated June 14, 2021 to the FDA IR, below are the relevant study days for Patient LUM001-304-(b) (6) from the initiation of randomized treatment to when maralixibat dosing was resumed. Patient LUM001-304-(b) (6) was randomized to the placebo arm, and this randomized treatment was interrupted 2 days prior to the end of the RWD period.

- Day 127 to 152: On randomized treatment (placebo)
- Day 153 to 155: Randomized treatment (placebo) interrupted because of an SAE
- Day 156 to 161: Randomized treatment (placebo) resumed
- Day 162 to 252: Treatment interruption at Applicant's request because of an SAE
- Day 253: Maralixibat dosing resumed

Table 9 lists the study days from which the ItchRO data for Patient LUM001-304-(b) (6) were used for the Applicant's main and sensitivity analyses during the RWD period according to the strategy described in the SAP. The Applicant's main analyses were based on the timing of the Week-22 study visit on Day 252, which was several months later than planned as a result of the patient's SAE.

Table 9. Patient LUM001-304-(b) (6): Study Days for Analysis Weeks During the RWD Period

Analysis Week	Applicant's Main Analyses (FDA Sensitivity Analysis)	Applicant's Sensitivity Analysis (FDA Main Analysis)
Week 19	224-230	127-133
Week 20	231-237	134-140
Week 21	238-244	141-147
Week 22	245-251	148-154

Source: Applicant's response (dated June 14, 2021) to the FDA's Information Request.
Abbreviations: FDA, Food and Drug Administration; RWD, randomized withdrawal

The Applicant's main analyses use data from when the patient had been off randomized study product for over 60 days; the meaningfulness of including these data in the analysis of the RWD period is unclear. Additionally, the ItchRO assessments were evaluated twice daily, regardless of study visit date, so it is not necessary to anchor the days used for the analysis of pruritus to the Week-22 study visit date.

The FDA considers the treatment policy strategy (FDA 2021) to be a more meaningful approach for evaluating the RWD period than the Applicant's main strategy. In this case, the treatment policy strategy uses the data from the four consecutive weeks after the initiation of randomized treatment (maralixibat or placebo), regardless of any subsequent intercurrent events, such as treatment discontinuation or interruption. This aligns with the Applicant's sensitivity analysis using the study days in Table 9.

For the reasons above and because there was no prespecification of a method for handling treatment discontinuation or interruption in the analysis, the FDA will present the main pruritus

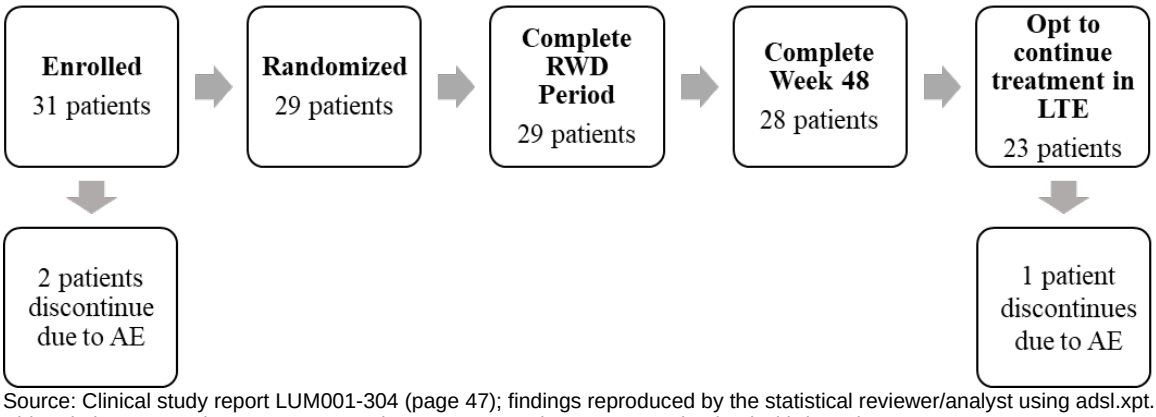
results using the treatment policy estimand. The FDA will present sensitivity analyses using the Applicant's main analysis methodology for handling the treatment interruption of Patient LUM001-304-(b) (6).

6.2.1.4. Results of Analyses, Study LUM001-304

Patient disposition is presented in Figure 5. A total of 31 patients was enrolled and treated with maralixibat in the initial 18-week OL period. Two (6%) of the thirty-one patients discontinued maralixibat treatment and the study during this open-label treatment period because of an AE. These two patients are included in subsequent efficacy analyses because they were not randomized in the RWD period.

The remaining 29 patients were randomized and completed the RWD period. After the re-initiation of open-label treatment after Week 22, one additional patient discontinued maralixibat treatment due to an AE. After the initially planned 48-week treatment period, 23 patients/caregivers opted to continue treatment under Protocol Amendment 3 in the LTE period.

Figure 5. Patient Disposition, Study LUM001-304



Source: Clinical study report LUM001-304 (page 47); findings reproduced by the statistical reviewer/analyst using adsl.xpt.
Abbreviations: AE, adverse event; LTE, long-term extension; RWD, randomized withdrawal

Patients were enrolled at nine sites: three sites in France, two sites in Australia, and one site in each of Belgium, Spain, Poland, and the United Kingdom. All enrolled patients had the JAGGED1 mutation.

Table 10 presents the baseline demographic and clinical characteristics of patients in Study LUM001-304 included in each period: the 31 patients in the initial OL treatment period (Weeks 1 to 18), the 29 patients by treatment arm randomized in the RWD period (Weeks 19 to 22), and the 29 patients who continued in the subsequent OL treatment period (Weeks 23 to 48). All characteristics in Table 10 were measured at baseline, i.e., prior to initiation of any study treatment.

The Applicant's submission did not contain race or ethnicity data for patients in Study LUM001-304, so summaries of these demographics are omitted from Table 10. In response to an IR from the FDA on March 2, 2021, the Applicant provided the following explanation for not collecting race and ethnicity data in Study LUM001-304:

> *"collection of race and ethnicity in France was prohibited according to data protection regulation Loi n° 78-17 du 6 janvier 1978 relative à l'informatique, aux fichiers et aux libertés, Article 6… In addition, at that time, a Canadian site ethics committee requested*

justification to collect race. Therefore, the study sponsor at the time decided to refrain from collection of race information in Study LUM001-304."

Table 10. Baseline (Pretreatment) Demographic and Clinical Characteristics, Study LUM001-304

	Open-Label Weeks 1-18	Randomized Withdrawal Weeks 19-22		Open-Label Weeks 23-48
Characteristic	**Maralixibat (N=31)**	**Maralixibat (N=13)**	**Placebo (N=16)**	**Maralixibat (N=29)**
Sex, n (%)				
Male	19 (61)	9 (69)	10 (63)	19 (66)
Female	12 (39)	4 (31)	6 (38)	10 (34)
Age, years				
Mean (SD)	5.4 (4.2)	5.4 (5.0)	5.8 (3.8)	5.6 (4.3)
Median (minimum, maximum)	4 (1, 15)	4 (1, 15)	5 (1, 14)	5 (1, 15)
Age group, n (%)				
<2 years	6 (19)	3 (23)	2 (13)	5 (17)
2 to 4 years	9 (29)	5 (38)	3 (19)	8 (28)
5 to 8 years	9 (29)	2 (15)	7 (44)	9 (31)
9 to 12 years	4 (13)	1 (8)	3 (19)	4 (14)
13 to 18 years	3 (10)	2 (15)	1 (6)	3 (10)
Country of participation, n (%)				
Australia	9 (29)	5 (38)	4 (25)	9 (31)
Belgium	5 (16)	1 (8)	2 (13)	3 (10)
France	9 (29)	3 (23)	6 (38)	9 (31)
Spain	3 (10)	2 (15)	1 (6)	3 (10)
Poland	2 (6)	0	2 (13)	2 (7)
United Kingdom	3 (10)	2 (15)	1 (6)	3 (10)
ItchRO(Obs) weekly average severity (Item 1), pretreatment				
Worst daily, mean (SD)	3.1 (0.5)	3.1 (0.4)	3.1 (0.5)	3.1 (0.5)
Morning, mean (SD)	2.9 (0.5)	2.9 (0.5)	2.9 (0.6)	2.9 (0.5)
Evening, mean (SD)	2.8 (0.6)	2.7 (0.7)	2.9 (0.5)	2.8 (0.6)
Average daily, mean (SD)	2.9 (0.5)	2.8 (0.4)	2.9 (0.5)	2.9 (0.5)
Serum bile acids, µmol/L				
Mean (SD)	283 (211)	318 (234)	250 (197)	280 (213)
Median (minimum, maximum)	276 (20, 748)	335 (31, 748)	196 (20, 583)	276 (20, 748)
Aspartate aminotransferase, U/L				
Mean (SD)	168 (76)	172 (76)	147 (61)	158 (68)
Median (minimum, maximum)	161 (32, 350)	183 (52, 350)	135 (32, 296)	158 (32, 350)
Alanine aminotransferase, U/L				
Mean (SD)	181 (109)	218 (150)	147 (55)	179 (112)
Median (minimum, maximum)	171 (41, 626)	196 (41, 626)	144 (54, 249)	164 (41, 626)
Gamma glutamyl transferase, U/L				
Mean (SD)	508 (389)	614 (482)	404 (300)	498 (399)
Median (minimum, maximum)	419 (86, 1545)	463 (86, 1545)	311 (99, 1021)	386 (86, 1545)
Total bilirubin, mg/dL				
Mean (SD)	6.1 (5.8)	6.5 (6.6)	4.8 (4.3)	5.6 (5.4)
Median (minimum, maximum)	4.6 (0.4, 20.5)	4.6 (0.5, 20.5)	2.9 (0.4, 13.4)	3.8 (0.4, 20.5)

Source: Clinical study report LUM001-304 (pages 49, 52, 54); findings reproduced and/or supplemented by the statistical reviewer using the adsl.xpt and adqs.xpt datasets.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); SD, standard deviation

Observer-Reported Pruritus Results: Randomized Withdrawal Period

Subsequent results for the 29 randomized patients are presented by randomized treatment group, because this enables valid, randomized treatment comparisons. Because randomized treatment

was initiated after Week 18, analyses of randomized patients estimate a treatment effect for the population of patients able to complete 18 weeks of OL treatment. The two patients who could not complete 18 weeks of OL treatment due to AE are included in analyses of safety but are not included in comparative analyses of efficacy.

Overall, there were minimal missing data for the weekly averages during the RWD phase. Only one randomized patient (maralixibat group) had a missing weekly average morning score at Week 22, and no patients had missing weekly average scores at Week 22 when evaluating the evening, worst daily, and average daily scores. There were a few randomized patients with missing weekly scores at Week 19, but no missing weekly scores at baseline and Weeks 18, 20, and 21 (refer to Table 133 in Section III.16.3 for details).

Focusing on the weekly averages of the worst daily scores (the largest of the morning and evening scores each day) as specified in the protocol (refer to Section 6.2.1.3), Table 11 presents summaries at baseline and results for Week 22, change from Week 18 to Week 22, and change from baseline to Week 22 averages of the worst daily ItchRO(Obs) scratching severity scores.

Results are based on ANCOVA models adjusting for the corresponding baseline measure: study baseline (pretreatment) and Week 18 (pre-randomized treatment). That is, the analysis of change from baseline to Week 22 adjusts for the average of the worst daily scores in the week before study baseline and the analysis of the change from Week 18 to Week 22 adjusts for the Week 18 average of the worst daily scores. Both sets of results support a beneficial treatment effect of remaining on maralixibat for 22 weeks compared to switching to placebo at Week 18, but have differing interpretations, as described below.

Change from Week 18 to Week 22

After 18 weeks of OL treatment, this compares the change in pruritus scores when staying on treatment versus discontinuing treatment for 4 weeks.

The results show that patients randomized to maralixibat on average had only a slight increase (0.2) in weekly average pruritus scores from Week 18 to Week 22, whereas patients randomized to placebo on average had a large increase (1.6) in weekly average pruritus scores during this RWD period. Patients randomized to placebo had greater worsening in pruritus on average during the RWD period than the patients randomized to maralixibat.

Change from Baseline to Week 22

This compares the change in pruritus scores when staying on continuous maralixibat treatment for 22 weeks (first 18 weeks are OL) versus OL maralixibat treatment for 18 weeks followed by a 4-week treatment interruption. This analysis is conditional on patients' ability to complete an initial 18 weeks of OL treatment.

The results demonstrate that patients randomized to maralixibat during the RWD period on average had a larger decrease (-1.5) in weekly average pruritus scores, equating to greater improvement in pruritus, from baseline to Week 22 compared to patients randomized to placebo during the RWD period (-0.1). Patients randomized to placebo on average had similar weekly average pruritus scores at baseline and at Week 22 after 4 weeks of treatment withdrawal.

The model-based 95% confidence intervals for the treatment differences exclude 0, and the results support the conclusion that continued treatment with maralixibat for 22 weeks has a

beneficial effect compared to switching to placebo after Week 18. Sensitivity analyses using the Applicant's strategy to handle the treatment interruption of Patient LUM001-304-(b) (6) yielded similar results (see Table 134 in Section III.16.3).

Table 11. Weekly Average of Worst Daily ItchRO(Obs) Scratching Severity Scores in the RWD Period, Treatment Policy Strategy, Study LUM001-304

ItchRO(Obs) Item 1 Variable	Maralixibat (N=13)	Placebo (N=16)	LS Mean Difference
Week 18 to Week 22			
Week 18[a], mean (SE)	1.5 (0.2)	1.3 (0.2)	NA
Week 22, LS mean (95% CI)	1.6 (1.1, 2.1)	3.0 (2.6, 3.5)	
Change, LS mean (95% CI)	0.2 (-0.3, 0.7)	1.6 (1.2, 2.1)	-1.4 (-2.1, -0.8)
Baseline to Week 22			
Baseline, mean (SE)	3.1 (0.1)	3.1 (0.1)	NA
Week 22, LS mean (95% CI)	1.6 (1.1, 2.2)	3.0 (2.5, 3.4)	
Change, LS mean (95% CI)	-1.5 (-2.0, -1.0)	-0.1 (-0.6, 0.3)	-1.3 (-2.0, -0.6)

Source: Reviewer's analysis using the Applicant-submitted dataset adqs2.xpt.
Results are based on ANCOVA models adjusted for Week 18 or baseline.
[a] Week-18 results are from the open-label treatment period and may be subject to bias because patients and caregivers were aware that patients were receiving active treatment.
Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; ItchRO(Obs), Itch Reported Outcome (Observer); LS, least squares; RWD, randomized withdrawal

Results were similar when evaluating weekly summaries of different daily summaries, i.e., using morning scores, evening scores, and the averages of the morning and evening scores, as presented in Table 16 in Section 6.3.2 (see Table 135 in Section III.16.3 for similar results using the Applicant's strategy to handle the treatment interruption of Patient LUM001-304-(b) (6)).

Observer-Reported Pruritus Results: 48-Week Study Period

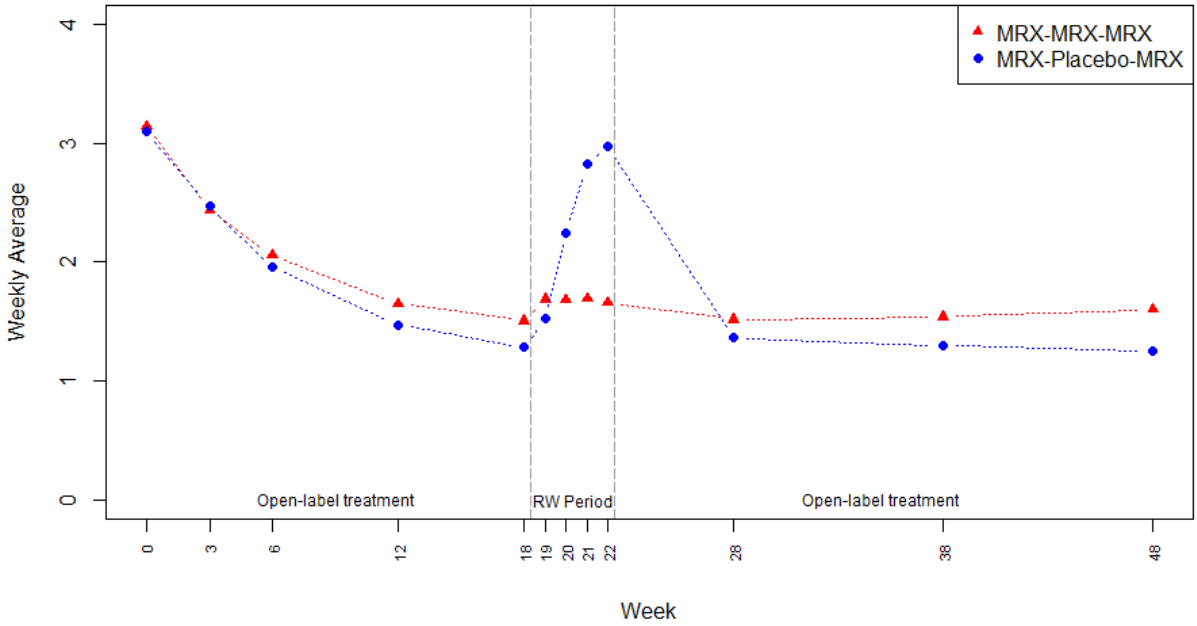
The results presented above evaluate pruritus during the double-blind, RWD period. Figure 6 shows the weekly averages of the worst daily ItchRO(Obs) scratching severity scores at key weeks during the 48-week study, including the open-label periods. These results are based on observed data only, with no imputation for missing data. There was minimal missing data with one patient in each of the maralixibat and placebo groups with missing Week-19 averages, one patient in the placebo group with a missing Week-28 average, and one patient in the maralixibat group with a missing Week-48 average.

Results are presented separately for the randomized treatment groups with different treatments during the RWD period; however, patients in both treatment groups were treated with maralixibat according to the same dosing schedule during the first 18 weeks of the study. Any differences between the trajectories during this initial OL treatment period may be due to the small sample sizes and random variations in patients' outcomes.

During the OL treatment periods, caregivers and patients are aware that the patient is receiving active maralixibat treatment, which may bias the results for this observer-reported outcome during these OL treatment periods. Therefore, these results during the OL treatment periods should be interpreted with caution. The trends show a large decrease in pruritus scores in the first 18 weeks of OL treatment. However, patients may naturally have episodic pruritus symptoms, where pruritus is severe during some periods and milder during others, irrespective of treatment. As a result of these natural variations and the lack of blinding and a comparator arm in the initial OL treatment period, the magnitude of the treatment effect when initiating maralixibat cannot be quantified.

In the OL treatment period following the RWD period (Weeks 22 to 48), pruritus improvement was observed in patients randomized to the placebo arm during the RWD after re-initiation of maralixibat treatment. On average, these patients returned to the same level of pruritus improvement as in the patients randomized to maralixibat during the RWD period (with no interruption of maralixibat treatment). These results may also be subject to bias due to the OL study design; however, the trend of these results may be informative to patients, caregivers, and prescribers in understanding the possible impact of maralixibat on pruritus if a treatment interruption is needed and treatment is subsequently re-initiated.

Figure 6. Weekly Average of Worst Daily ItchRO(Obs) Scratching Severity Scores over Time, Treatment Policy Strategy (Observed Data), Study LUM001-304



Source: Reviewer's analysis using the Applicant-submitted dataset adqs2.xpt.
The results during the open-label treatment periods may be biased due to caregivers' and patients' knowledge that the patient was receiving active maralixibat treatment, and these results should be interpreted with caution.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; RW, randomized withdrawal

The patient-level trajectories over the 48-week study period in Figure 44 in Section III.16.3 show similar trends to the population-level trajectories in Figure 5.

Patient-Reported Outcome Results: Randomized Withdrawal Period

To support the main efficacy results based on the ItchRO(Obs), results on the ItchRO(Pt) were also evaluated. Participants 5 years of age and older were to assess their scratching on the ItchRO(Pt) (refer to Section 6.2.1). There were 16 patients, 5 randomized to maralixibat and 11 randomized to the placebo arm, who were 5 years of age or older at study baseline. Two of these patients randomized to the placebo arm did not assess the ItchRO(Pt): one patient was less than 5 years of age during the screening period (prebaseline), and one patient was not capable of understanding and completing the ItchRO, even with the help of a caregiver, in the opinion of the Principal Investigator. Therefore, results are presented for the 14 patients, 5 randomized to maralixibat and 9 randomized to placebo, who assessed the ItchRO(Pt).

The weekly averages of the worst daily ItchRO(Pt) scores during the RWD period are presented in Table 12. There were no missing data for these analyses. These results are supportive of the

observer-reported results (Table 11), and support the conclusion that continued treatment with maralixibat for 22 weeks has a beneficial effect compared to switching to placebo after Week 18. Sensitivity analyses using the Applicant's strategy to handle the treatment interruption of Patient LUM001-304-(b) (6) had similar results (Table 136 in Section III.16.3).

Table 12. Weekly Average of Worst Daily ItchRO(Pt) Scratching Severity Scores in the RWD Period, Treatment Policy Strategy, Study LUM001-304

ItchRO(Pt) Item 1 Variable	Maralixibat (N=5)	Placebo (N=9)	LS Mean Difference
Week 18 to Week 22			
Week 18*, mean (SE)	0.9 (0.3)	0.9 (0.3)	NA
Week 22, LS mean (95% CI)	0.8 (0, 1.7)	2.7 (2.1, 3.4)	
Change from Week 18 to Week 22, LS mean (95% CI)	-0.1 (-1.0, 0.7)	1.8 (1.1, 2.4)	-1.9 (-3.0, -0.9)
Baseline to Week 22			
Baseline, mean (SE)	2.9 (0.2)	3.1 (0.2)	NA
Week 22, LS mean (95% CI)	0.8 (0, 1.7)	2.7 (2.1, 3.4)	
Change from baseline to Week 22, LS mean (95% CI)	-2.2 (-3.1, -1.4)	-0.3 (-0.9, 0.4)	-1.9 (-3.0, -0.9)

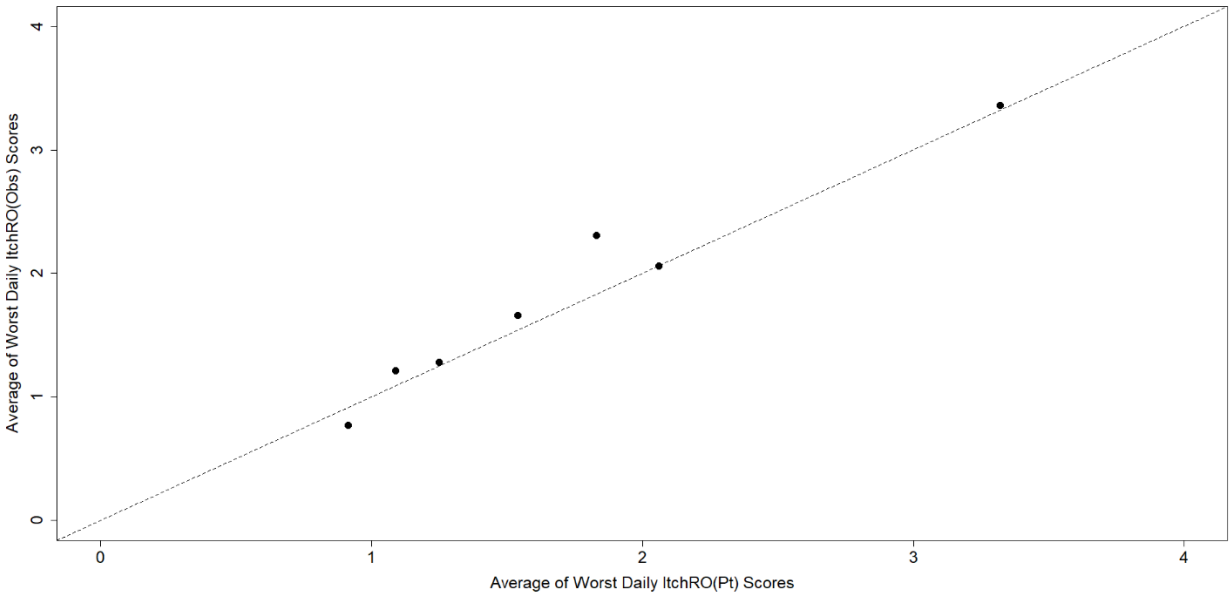
Source: Reviewer's analysis using the Applicant-submitted dataset adqs2.xpt.
Results are based on ANCOVA models adjusted for Week 18 or baseline. There were no missing weekly observations at Week 18 or Week 22.
* Week 18 results are from the open-label treatment period and may be subject to bias, as patients and caregivers were aware that patients were receiving active treatment.
Abbreviations: CI, confidence interval; ItchRO(Pt), Itch Reported Outcome (Patient); LS, least squares; RWD, randomized withdrawal; SD, standard deviation; SE, standard error

Comparability of Patient and Caregiver Pruritus Assessments: RWD Period

As shown in Table 12, a total of 14 patients completed the ItchRO(Pt) either themselves or with the assistance of their caregiver. Of these, a total of seven patients (four in the placebo arm and three in the maralixibat arm), aged 9 years and older, independently completed the ItchRO(Pt), i.e., without the help of their caregiver. The FDA conducted post hoc descriptive analyses to evaluate the relationship between patient and caregiver assessments of pruritus severity among the seven patients with both ObsRO and independently self-reported patient-reported outcome score data during the randomized withdrawal period (i.e., Week 18 to Week 22). Patients were pooled across study arms and missing data were not imputed.

Figure 6 shows the weekly average of worst daily ItchRO(Obs) item 1 score (hereafter referred to as ItchRO(Obs) score) from the caregiver's perspective plotted against the average of worst daily ItchRO(Pt) item 1 score (hereafter referred to as ItchRO(Pt) score) from the patient's perspective. Visual inspection shows general alignment between the scores, suggesting that ItchRO(Obs) scores may be considered a reasonable representation of patients' pruritus (scratching). However, there is some uncertainty surrounding these findings because of the very small number of patients (n=7) with both ObsRO and independently self-reported patient-reported outcome score data available during the RWD period.

Figure 7. Scatterplot of Weekly Average of Worst Daily ItchRO(Obs) Scores by ItchRO(Pt) Score During the Randomized Withdrawal Period, Study LUM-304



Source: PFSS Reviewer's figure using the Applicant-submitted dataset adqs2.xpt.
Based on seven patients with both ObsRO and independently self-reported PRO scores during the randomized withdrawal period.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); ItchRO(Pt), Itch Reported Outcome (Patient); PFSS, Patient-Focused Statistical Support

Table 13 (morning) and Table 14 (evening) show crosstabulations of patients' responses (n=7) to ItchRO(Pt) item 1 and caregiver's responses to ItchRO(Obs) item 1 during the RWD period. Of the caregivers' responses, 79% to 98% matched those of the patients for the AM diary; 58% to 94% of the caregivers' responses matched those of the patients for the PM diary, and no ItchRO(Obs) and ItchRO(Pt) assessments differed by more than one category, further supporting the use of the ItchRO(Obs) as a measure of pruritus (scratching).

Table 13. Crosstabulation of Patient and Caregiver Responses to AM ItchRO During the Randomized Withdrawal Period, Study LUM-304

	Caregiver (ItchRO[Obs])				
Patient (ItchRO[Pt])	**0**	**1**	**2**	**3**	**4**
0	40 (98%)	1 (2%)	0	0	0
1	3 (4%)	72 (86%)	9 (11%)	0	0
2	0	3 (9%)	27 (84%)	2 (6%)	0
3	0	0	1 (4%)	19 (79%)	4 (17%)
4	0	0	0	1 (2%)	45 (98%)

Source: PFSS Reviewer's table using the Applicant-submitted dataset adqs2.xpt.
ItchRO(Obs) response options: 0 = none observed or reported, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe.
ItchRO(Pt) response options: 0 = I didn't feel itchy, 1 = I felt a little bit itchy, 2 = I felt pretty itchy, 3 = I felt very itchy, 4 = I felt very, very itchy.
Based on seven patients with both ObsRO and independently self-reported patient-reported outcome score data and a total of 227 AM ItchRO responses during the randomized withdrawal period.
Abbreviations: ItchRO, Itch Reported Outcome; ItchRO(Obs), Itch Reported Outcome (Observer); ItchRO(Pt), Itch Reported Outcome (patient); PFSS, Patient-Focused Statistical Support

Table 14. Crosstabulation of Patient and Caregiver Responses to PM ItchRO During the Randomized Withdrawal Period, Study LUM-304

	Caregiver (ItchRO[Obs])				
Patient (ItchRO[Pt])	**0**	**1**	**2**	**3**	**4**
0	50 (91%)	5 (9%)	0	0	0
1	12 (13%)	72 (77%)	9 (10%)	0	0
2	0	3 (12%)	17 (65%)	6 (23%)	0
3	0	0	2 (17%)	7 (58%)	3 (25%)
4	0	0	0	3 (6%)	48 (94%)

Source: PFSS Reviewer's table using the Applicant-submitted dataset adqs2.xpt.
ItchRO(Obs) response options: 0 = none observed or reported, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe.
ItchRO(Pt) response options: 0 = I didn't feel itchy, 1 = I felt a little bit itchy, 2 = I felt pretty itchy, 3 = I felt very itchy, 4 = I felt very, very itchy.
Table is based on seven patients with both ObsRO and independently self-reported patient-reported outcome score data and a total of 237 PM ItchRO responses during the randomized withdrawal period.
Abbreviations: ItchRO, Itch ReportedOutcome; ItchRO(Obs), Itch Reported Outcome (Observer); ItchRO(Pt), Itch Reported Outcome (Patient); PFSS, Patient-Focused Statistical Support

Subgroup Analyses: Observer-Reported Pruritus Results

Table 15 presents efficacy results by sex and age subgroups for Week 22, the change from baseline to Week 22, and the change from Week 18 to Week 22 averages of the worst daily ItchRO(Obs) scratching severity scores. The Applicant did not submit race or ethnicity data, so these subgroup results are not presented. Model-based estimates and 95% confidence intervals (CIs) are not presented because of the small sample sizes of the subgroups.

Subgroup results by sex are consistent with the overall results. It is difficult to make conclusions regarding the potential of differential efficacy across age subgroups due to small sample sizes; however, older maralixibat-treated patients tended to have better outcomes than younger maralixibat-treated patients. Other than the <2 years of age subgroup, trends are in favor of the maralixibat arm compared to the placebo arm based on the sample mean estimates. Interpretation of the results is limited by the small sample sizes in both arms.

Table 15. Subgroup Results for Weekly Average of Worst Daily ItchRO(Obs) Scratching Severity Scores in the RWD Period, Treatment Policy Strategy, Study LUM001-304

ItchRO(Obs) Item 1 Variable and Subgroup	**Maralixibat**		**Placebo**	
	N	**Mean (SE)**	**N**	**Mean (SE)**
Week 22				
Overall	13	1.7 (0.3)	16	3.0 (0.2)
Sex				
Male	9	1.7 (0.3)	10	3.1 (0.2)
Female	4	1.6 (0.6)	6	2.8 (0.4)
Age group				
<2 years	3	3.0 (0.1)	2	2.7 (0.7)
2 to 4 years	5	1.7 (0.4)	3	3.6 (0.4)
5 to 8 years	2	0.9 (0.4)	7	2.8 (0.3)
9 to 18 years	3	0.7 (0.3)	4	3.0 (0.4)

ItchRO(Obs) Item 1 Variable and Subgroup	Maralixibat N	Maralixibat Mean (SE)	Placebo N	Placebo Mean (SE)
Change from baseline to Week 22				
Overall	13	-1.5 (0.3)	16	-0.1 (0.2)
Sex				
Male	9	-1.5 (0.4)	10	-0.1 (0.2)
Female	4	-1.5 (0.6)	6	-0.1 (0.3)
Age group				
<2 years	3	-0.4 (0.1)	2	-0.2 (0.9)
2 to 4 years	5	-1.4 (0.6)	3	0.1 (0.1)
5 to 8 years	2	-2.1 (0.4)	7	-0.1 (0.2)
9 to 18 years	3	-2.4 (0.6)	4	-0.3 (0.5)
Change from Week 18 to Week 22				
Overall	13	0.2 (0.2)	16	1.7 (0.3)
Sex				
Male	9	0.1 (0.3)	10	1.8 (0.3)
Female	4	0.3 (0.1)	6	1.5 (0.6)
Age group				
<2 years	3	0.7 (0.1)	2	0.4 (0.2)
2 to 4 years	5	0.1 (0.2)	3	2.3 (0.2)
5 to 8 years	2	0.4 (0.1)	7	1.9 (0.3)
9 to 18 years	3	-0.5 (0.7)	4	1.5 (0.9)

Source: Reviewer's analysis using the Applicant-submitted dataset adqs2.xpt.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); RWD, randomized withdrawal; SE, standard error

6.3. Key Review Issues Relevant to Evaluation of Benefit

6.3.1. Regulatory Framework for Establishing Substantial Evidence of Effectiveness

Issue

Study LUM001-304 is one adequate and well-controlled investigation. Is there adequate confirmatory evidence to meet the substantial evidence standard?

Background

ALGS is a rare disease, with a prevalence of 1:30,000 to 1:70,000 (Danks et al. 1977; Leonard et al. 2014).

The development program consisted of three randomized, placebo-controlled studies assessing the effect of maralixibat on pruritus symptoms in children with ALGS. The two initial, supportive studies (LUM001-301 and LUM001-302) were randomized, placebo-controlled studies of 13-week duration. The maximum dose tested in these two studies was 280 mcg/kg/day (with a majority of subjects receiving doses <280 mcg/kg/day). Although there was some modest numerical improvement in ItchRO(Obs) in the treatment arms of these two studies, no significant benefit on pruritus was demonstrated in LUM001-301 and LUM001-302. Therefore, substantial evidence of efficacy is based on the effect on pruritus in LUM001-304, along with confirmatory evidence based on supportive mechanistic data (effect on sBAs and animal model data).

Study LUM001-304 was a global, multicenter study. Thirty-one patients were enrolled at nine study centers in five countries in Europe (Belgium, France, Poland, Spain, and the United Kingdom) and Australia.

The Applicant conducted a single, OL study with a double-blind, RWD period demonstrating a persuasive treatment effect on relief of pruritus in patients with ALGS. Patients could roll over to an OL extension after completing Study LUM001-304.

Assessment

Study LUM001-304 Results and Review of Evidence

Primary evidence of effectiveness from Study LUM001-304 supporting approval:

- Evidence from a clinically meaningful endpoint that is a direct measure of how a patient feels and functions. Severe pruritus is a detrimental symptom for pediatric patients with ALGS and is an indication for liver transplantation. The FDA's main analysis was based on endpoints evaluating weekly averages of the worst daily scores of the ItchRO(Obs). Due to the lack of prespecification of a single primary pruritus endpoint, the FDA investigated multiple pruritus endpoints (Section 6.2) using the ItchRO(Obs). Supportive analyses were conducted on the basis of the patient-reported outcome, ItchRo(Pt), in patients of sufficient age and able to self-report their itching. These analyses demonstrated a consistent benefit of continuing maralixibat for 22 weeks, when compared with switching to placebo after Week 18.

Each of the nine centers contributed one to six patients, and the efficacy results were not driven by a single patient or a small subset of patients (Figure 45).

Supportive evidence is provided by the following mechanistic findings:

- Reduction of sBA levels was observed in maralixibat-treated patients. Mean sBA levels at baseline were 283 µmol/L in the 31 patients enrolled and 280 mmol/L (range 20 to748 µmol/L) in the 29 patients randomized in the RWD period in LUM001-304 (ULN for sBA is <8.5 mmol/L). By the end of the OL treatment period (Week 18), there was a mean (standard error) decrease of 88 (22) µmol/L in the 29 randomized patients. At Week 22, the mean (standard error) change from baseline in sBA level was -94 (36) µmol/L in the maralixibat arm and -3 (31) mmol/L in the placebo arm, for a treatment difference of -91 µmol/L (95% CI -187, 5 µmol/L) based on an ANCOVA model with treatment group as a fixed effect and baseline value as a covariate.

- During the RWD period, the return to baseline in pruritus symptoms, on average, was mirrored by a return to baseline sBA level, on average, in the placebo arm. In contrast, the sBA level and pruritus symptoms did not markedly change on average in the maralixibat-treated arm from Week 18 to 22. Although the pathophysiology of pruritus is multifactorial and not fully understood, elevations in sBA levels, likely reflecting an increase in a contributing pruritogen (Kremer et al. 2014), are typically observed in patients with pruritus.

- Nonclinical evidence of proof-of-concept. In the rat pBDL model of cholestasis (Study #(b) (4)2013RES01 and #R7311M-SHP625), maralixibat alone at oral doses of 0.3, 1, and

10 mg/kg/day or a combination of maralixibat (1 mg/kg/day) and UDCA (1 mg/kg/day) reduced sBAs, ALP, AST, ALT, GGT, and total bilirubin after 14 days of treatment. The combined treatment resulted in slightly greater improvements in these biomarkers than maralixibat or UDCA alone (Section III.13.2.1).

Conclusions

The main source of evidence to support the approval is from one adequate and well-controlled study, i.e., Study LUM001-304.

The primary pruritus endpoint is related to how a patient feels and functions. Maralixibat demonstrated statistically persuasive results in improvement of pruritus across different pruritus endpoints and sensitivity analyses.

In addition to the clinical endpoint of improvement in pruritus, the reduction in sBA levels further supported the mechanism of action and provided supportive evidence for efficacy. In the pivotal study, patients with sBA levels >3× ULN for age were enrolled and 74% of patients had sBA levels >10× ULN. Upon maralixibat treatment, sBA levels substantially decreased (although only one patient achieved a normal sBA) and remained lower over time. In the RWD period, sBAs returned to pretreatment levels in the subjects on placebo.

The nonclinical studies are supportive of the mechanistic rationale.

Therefore, the FDA accepts a single adequate and well-controlled study with confirmatory evidence as sufficient to support a recommendation for approving maralixibat for “treatment of pruritus” in ALGS, with pruritis being a severely debilitating aspect of the disease for which there is no FDA-approved therapy.

6.3.2. Randomized Withdrawal Design

Issue

Study LUM001-304 is a randomized withdrawal study. Is a randomized withdrawal study design adequate to demonstrate a beneficial effect on pruritus in ALGS?

Background

Study LUM001-304, which compared the effect of 400 mcg/kg/day with placebo during an RWD period after an 18-week OL treatment period, is the single adequate and well-controlled study that is used to support this application based on a beneficial effect on pruritus symptoms.

Assessment

There are several potential concerns with randomized withdrawal study designs:

- There is a potential for a “rebound” or exaggerated effect of the underlying symptom in the placebo arm after the drug is withdrawn.
  - There is the potential that discontinuation of a drug could lead to a rebound or exaggerated effect of the underlying symptom above the baseline that would lead to the placebo arm having worse symptoms than at baseline, in turn exaggerating the benefit of the study drug. The return of both pruritus symptoms and sBA levels to

baseline (but not worse than baseline) indicates that a rebound effect was not present in the Study LUM001-304 results.

- In most randomized withdrawal designs, the randomized population for comparison during the placebo-controlled period is enriched for patients who have responded to the drug, which does not reflect the intended use population (lacks external validity).
  - Often, a RWD study design randomizes only the subgroup of patients who had an initial response and were tolerant of the study drug after a period of OL active treatment. This is unlikely to be an issue in Study LUM001-304. All patients who remained in the study at the time of randomization were randomized in the RWD period; therefore, patients who were considered nonresponders in the initial OL treatment period were included in the efficacy analyses. Two of thirty-one patients withdrew from the study during the initial OL period, i.e., prior to Week 18, the randomized portion of the study. The reasons for withdrawal (subdural hemorrhage and staphylococcal infection of the hand) did not reflect intolerance to maralixibat or lack of efficacy (i.e., response of pruritus).
- Lack of ability to interpret the magnitude of the treatment effect on pruritus due to the placebo-response effect not being fully separated from the effect of study drug.
  - During the blinded, placebo-controlled randomized portion of the study, 100% of patients randomized to placebo had an increase in the ItchRO (Obs) with a mean ItchRO(Obs) score similar to baseline (Table 11). In the maralixibat arm, although the mean ItchRO(Obs) score did not return to baseline, it did numerically increase. In addition, over half of the patients who continued to receive maralixibat in a blinded fashion during the RWD period had an increase in the ItchRO(Obs) score, potentially due to uncertainty regarding whether patients were receiving study drug or placebo (Table 17 and Figure 44) during the 4-week period.

Conclusion

A primary limitation of this study design was the inability to assess the full magnitude of the effect on pruritus upon initiation of treatment. However, other potential limitations of RWD study designs did not apply in this setting. The randomized population used for efficacy analyses was not enriched for those who tolerated or responded to therapy and there was no evidence of a rebound or worsening of pruritus over baseline symptoms during the placebo-controlled period.

Although there were limitations with this study design, the magnitude of the differences between treatment and placebo arms during the RWD period, and the return of response to therapy with resumption of treatment in the placebo arm demonstrate that maralixibat has a beneficial effect on pruritus symptoms in patients with cholestatic disease from ALGS.

6.3.3. Uncertainty of Itch Reported Outcome Instrument (ItchRO[Obs]) Item 1

Issue

- The ItchRO(Obs) item 1 (Figure 4) combines “none observed” and “none reported” into a single response option of “0”, making data collected for this response option difficult to interpret.

- The ItchRO(Obs) item 1 (Figure 4) stem incorporates proximal impacts of itch, (i.e., sleep disturbances and irritability), resulting in a multibarreled concept item. This construction makes it unclear whether the caregivers' responses reflect exclusively the patients' itch-related symptoms.

Background

Sub-Issue 1

During the IND phase (IND 119917; June 18, 2019, type B EOP2 meeting), the FDA expressed concern that the version of ItchRO(Obs) administered in Study LUM001-304 was not optimized. Specifically, a previous version of ItchRO(Obs) developed and revised in accordance with the FDA's advice was not used in Study LUM001-304. The Applicant noted that the modification of the ItchRO(Obs) occurred after Study LUM001-304 enrollment was completed; therefore, the optimal version of ItchRO(Obs) could not be implemented. A major concern of the version of ItchRO(Obs) administered in Study LUM001-304 centered on the response option "0" on item 1. The FDA questioned the interpretability of the data collected from ItchRO(Obs) item 1, given that the response option "0" combines the options "none observed" and "none reported," making the two scenarios indistinguishable.

Sub-Issue 2

The ItchRO(Obs) item 1 stem "Based on observations or what your child told you about his/her itching, how severe were your child's itch-related symptoms (rubbing, scratching, skin damage, sleep disturbances or irritability)…," includes other concepts in addition to itch (i.e., proximal impacts of itch; sleep disturbances and irritability), which results in a multibarreled item. It was unclear how the caregivers' responses to this item are influenced by the patients' proximal impacts.

Assessment

Item 2 of the ItchRO(Obs) (refer to Figure 39 and Figure 40 in Section III.16.1.5) asked caregivers to select all of the symptoms that contributed to their answer to item 1. Specifically, the response options of item 2 include "Child-reported itching," "Observed difficulty falling asleep or staying asleep (sleep disturbance)," "Observed rubbing or scratching," "Observed new or worsening marks on the skin due to rubbing or scratching," and "Observed fussiness or irritability."

Sub-Issue 1

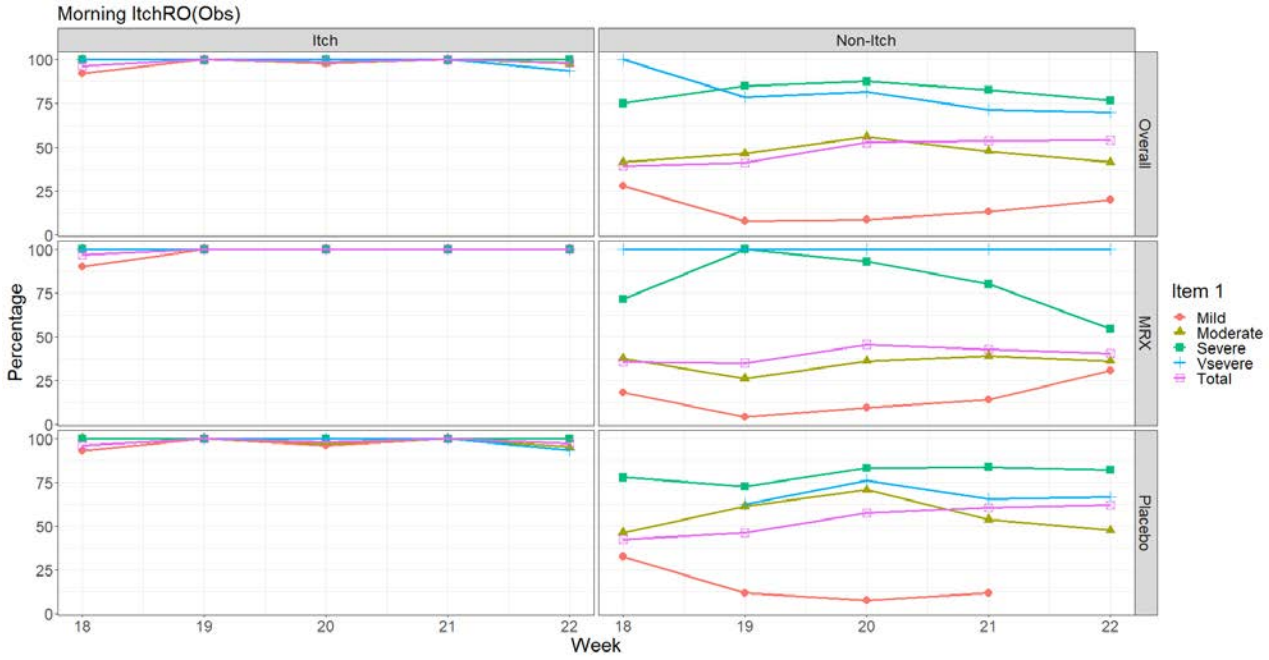
If the caregiver selected response option "0 = none observed or reported" for ItchRO(Obs) item 1, item 2 was not shown. Therefore, responses to item 2 could not be used to confirm whether the response option "0" for item 1 corresponded to a lack of itch-related symptoms observed by the caregivers. However, as shown in Table 13 and Table 14, 98% and 91% of caregivers' "0" responses (i.e., "None observed or reported") matched patients' "0" responses (i.e., "I didn't feel itchy") on the ItchRO(Obs) AM and PM eDiaries, respectively. Despite the small sample size (n=7), the high concordance between the patients' and caregivers' pruritus assessments provided support for the interpretation of the response option "none observed or reported" of the ItchRO(Obs) item 1. To further understand the extent to which the "0" response

option of item 1 impacted the ItchRO(Obs) scores, the frequency of these responses during the randomized withdrawal period were examined. In the treatment group, 23% of nonmissing responses to the ItchRO(Obs) were "0" compared to 13% in the placebo group.

Sub-Issue 2

When caregivers selected any response options to item 1 other than "none observed or reported" (i.e., "mild," "moderate," "severe," or "very severe"), item 2 of the ItchRO (Obs) became available. To better understand whether itch-related symptoms are exclusively driving caregivers' responses to item 1 (i.e., to ensure that responses were not driven by proximal impacts of itch, such as sleep disturbances and irritability), the Patient-Focused Statistical Support and Division of Clinical Outcome Assessment teams asked the Applicant to provide counts for each of the response options in item 2 aggregated based on the response category chosen for item 1. Using data submitted in the Applicant's March 31, 2021 response to the FDA's March 26, 2021 information request, the Patient-Focused Statistical Support reviewer compared the percentages of itch-related symptom responses (i.e., "Child reported itching," "Observed rubbing or scratching," and "Observed new or worsening marks on the skin due to rubbing or scratching,") and the percentage of symptom responses related to proximal impacts of itch (i.e., non-itch related symptoms including "Observed difficulty falling asleep or staying asleep (sleep disturbance)" and "Observed fussiness or irritability"). Figure 8 shows the percentage of aggregated itch-related and aggregated non-itch related symptoms reported in ItchRO(Obs) item 2 based on the response category chosen for item 1, by treatment arm as well as for the overall sample (pooled across treatment arms) during the RWD period. Because the pattern is similar for both AM and PM ItchRO(Obs) scores, only the AM scores are presented. Itch-related symptoms are very commonly (>80%) reported across all item 1 severity levels, whereas non-itch-related symptoms are less commonly reported, with the most frequently reported non-itch-related symptoms associated with "severe" or "very severe" responses to item 1.

Figure 8. Percentages of Aggregated Itch-Related and Aggregated Non-Itch-Related Symptoms Reported in Item 2 by Item 1 Response Category on the AM ItchRO(Obs), Study LUM001-304



Source: PFSS Reviewer's Figure using the Applicant-submitted dataset adqs2.xpt.
Figure is based on ItchRO(Obs) AM eDiary data submitted by the Applicant in the March 31, 2021 response to the FDA's March 26, 2021 information request.
If caregiver selected "0=none observed or reported", item 2 was not shown.
The left panel, "itch," aggregates the ItchRO(Obs) item 2 symptom responses "Child reported itching," "Observed rubbing or scratching," and "Observed new or worsening marks on the skin due to rubbing or scratching." The right panel, "non-itch," aggregates the ItchRO(Obs) item 2 symptom responses "Observed difficulty falling asleep or staying asleep (sleep disturbance)" and "Observed fussiness or irritability."
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PFSS, Patient-Focused Statistical Support

Conclusion

The FDA's assessments concluded that ItchRO(Obs) item 1 was a reasonable measure of pruritus and data collected from item 1 could support a pruritus endpoint(s).

6.3.4. No Single Prespecified Pruritus Endpoint

Issue

There was no single prespecified primary pruritus endpoint or analysis and no prespecified methodology to account for testing multiple endpoints.

Background

To provide confidence in the results and conclusions of a clinical study, the endpoints, analysis methodology, and methodology to account for testing multiple endpoints should be prospectively specified (FDA 1998; FDA 2017). Study LUM001-304 was designed as a Phase 2 study and the protocol and SIAP that were created prior to unblinding of the study data did not specify a single primary pruritus endpoint or analysis. The SAP was finalized after the study data were unblinded.

The primary endpoint specified in the protocol was the mean change from Week 18 to 22 in fasting sBA levels in subjects who previously responded to maralixibat treatment, as defined by a reduction in sBA ≥50% from baseline to Week 12 or Week 18. The protocol listed pruritus outcomes for secondary endpoints that were not uniquely defined and stated that no adjustments would be made for testing multiple endpoints.

Assessment

Although there was no clear hierarchy of pruritus endpoints, the protocol prespecified evaluating weekly averages of the worst daily scores; therefore, the FDA's primary analyses focused on this summary of the data. To have a valid treatment comparison, the FDA's analyses focused on the RWD period. There was no missing data for the weekly average of the worst daily scores at baseline, Week 18, and Week 22.

To ensure that the pruritus efficacy results were not driven by a single choice of endpoint or analysis, the FDA also evaluated weekly averages of different daily summaries (morning, evening, and the average of the morning and evening scores) and different ways of accounting for treatment interruption.

Table 16 shows that results were similar when evaluating weekly averages of different daily summaries (i.e., morning score, evening score, and average of morning and evening scores) compared to the main analysis using the worst daily score (full results in Table 11 in Section 6.2.1). Table 135. in Section III.16.3 presents similar results using the Applicant's strategy to handle the treatment interruption of Patient LUM001-304-(b) (6). Results on the ItchRO(Obs) were supported by those on the ItchRO(Pt) (Table 12 in Section 6.2.1.4).

Table 16. Results for the Weekly Average of ItchRO(Obs) Scratching Severity Scores in the RWD Period Using Various Daily Summaries, Treatment Policy Strategy, Study LUM001-304

ItchRO(Obs) Item 1 Variable	Maralixibat (N=13)	Placebo (N=16)	LS Mean Difference
Change from baseline to Week 22, LS mean (95% CI)			
Worst daily score	-1.5 (-2.0, -1.0)	-0.1 (-0.6, 0.3)	-1.3 (-2.0, -0.6)
Morning score*	-1.3 (-1.9, -0.8)	-0.2 (-0.7, 0.3)	-1.1 (-1.9, -0.4)
Evening score	-1.3 (-1.8, -0.8)	-0.1 (-0.6, 0.3)	-1.2 (-1.8, -0.5)
Average of AM and PM scores	-1.3 (-1.8, -0.8)	-0.2 (-0.6, 0.3)	-1.2 (-1.8, -0.5)
Change from Week 18 to Week 22, LS mean (95% CI)			
Worst daily score	0.2 (-0.3, 0.7)	1.6 (1.2, 2.1)	-1.4 (-2.1, -0.8)
Morning score*	0.3 (-0.2, 0.8)	1.6 (1.1, 2.0)	-1.3 (-1.9, -0.6)
Evening score	0.2 (-0.2, 0.7)	1.6 (1.2, 2.0)	-1.4 (-2.0, -0.7)
Average of AM and PM scores	0.3 (-0.2, 0.7)	1.6 (1.2, 2.0)	-1.3 (-2.0, -0.7)

Source: Reviewer's analysis using Applicant-submitted dataset adqs2.xpt. Results for morning score are reproduced from the Clinical Study Report LUM001-304 Tables and Figures document (page 159, Table 14.2.1.7).
Results are based on an ANCOVA models adjusted for baseline or Week 18 respectively.
*LOCF was used to impute one missing morning score for Patient LUM001-304-(b) (6)
Abbreviations: CI, confidence interval; ItchRO(Obs), Itch Reported Outcome (Observer); LS, least squares; RWD, randomized withdrawal

Conclusion

The results from all analyses of pruritus support the conclusion that continued treatment with maralixibat for 22 weeks has a beneficial effect compared to switching to placebo after Week 18.

6.3.5. Evaluation of Clinically Meaningful Within-Patient Change in Pruritus

Issue

Study LUM001-304 did not include appropriate anchor scale(s) to support the evaluation of anchor-based clinically meaningful within-patient change in any pruritus endpoint(s) during the randomized withdrawal period.

Background

The Agency recommends the use of anchor-based methods to establish meaningful within-patient changes in clinical outcome assessment-based endpoint(s), although other methods (e.g., qualitative evidence) can be used (FDA 2019). The Applicant did not seek the FDA's advice on the selection of appropriate anchor scale(s) or other methods to support the evaluation of meaningful within-patient change (specifically for the RWD period) prior to data unblinding in the IND phase. As a result, no qualitative data were available to aid in the interpretation of pruritus endpoint(s) in Study LUM001-304. However, Study LUM001-304 did include two reference (anchor) measures: the caregiver impression of itch (CIC) and the Clinician Scratch Scale. In addition, given the randomized study withdrawal design of Study LUM-304 (Sections 6.2.1.1 and 6.3.2), anchor-based meaningful within-patient change analysis must be conducted using pruritus endpoint(s) data generated from the randomized withdrawal period, i.e., Week 18 to Week 22. Consequently, any anchor scale(s) assessing change and used to aid in the interpretation of meaningful change upon randomized withdrawal would need to have a recall period over Week 18 to Week 22. The CIC's recall period is "since the start of the study" to the time when the assessment is completed by the caregiver and, therefore, does not reference patient symptoms immediately prior to the RWD period. Specifically, the schedule of the CIC's assessment periods was Week 18, 22, and 48. Therefore, the "since the start of the study" until either the Week 18, 22 or 48 recall periods for change in itch-related symptoms do not match the recall period needed for interpretation of meaningful change over Week 18 to Week 22. The recall period "since the start of the study" to Week 22 for patients in the placebo group during the RWD period includes time periods where the patients are on active maralixibat treatment as well as placebo, leading to difficulties in interpretation. The Clinician Scratch Scale assesses the clinician's observation of scratching at the time of administration with essentially no past recall period. As discussed above, no such anchor measures to directly interpret meaningful within-patient change upon RWD were available in Study LUM001-304.

The Applicant conducted anchor-based analyses supplemented with empirical cumulative distribution function curves to evaluate meaningful within-patient change in multiple pruritus endpoint scores (e.g., average weekly AM and PM ItchRO(Obs) scores; also refer to Section 6.3.4) using the CIC and Clinician Scratch Scale as anchor scales at varying timepoints (i.e., Baseline to Week 18, Baseline to Week 48, Week 18 to Week 22, and Week 19 to Week 22). Although the Applicant followed the FDA's current methodological recommendation on the evaluation of meaningful change, the various analyses conducted by the Applicant could not be used to determine whether patients experienced a clinically meaningful change in pruritus for the following reasons: 1) There was a lack of appropriate anchor scale(s) data, as discussed above; and 2) ItchRO(Obs) and ItchRO(Pt) data collected during OL treatment periods (i.e.,

Baseline to Week 18, Week 22 to Week 48) are challenging to interpret due to the potential for bias caused by patients' and/or caregivers' knowledge of treatment assignment[1].

Assessment

As discussed in Section 6.3.4, the results from FDA's efficacy analyses of pruritus support the conclusion that continued treatment with maralixibat for 22 weeks has a beneficial effect compared to switching to placebo after Week 18. Given this consistent and notable efficacy finding, the Patient-Focused Statistical Support team concluded that anchor-based clinically meaningful within-patient change analysis was not needed to interpret the treatment effect in pruritus.

To further evaluate the magnitude of the treatment effect in pruritus and given the RWD design of Study LUM-304, it is of interest to examine the difference in the proportion of patients in the placebo group (i.e., discontinuing maralixibat) and in the treatment group (i.e., continuing maralixibat) who experienced a worsening (i.e., any numerical increase in pruritus scores from Week 18) in itch-related symptoms during the RWD period.

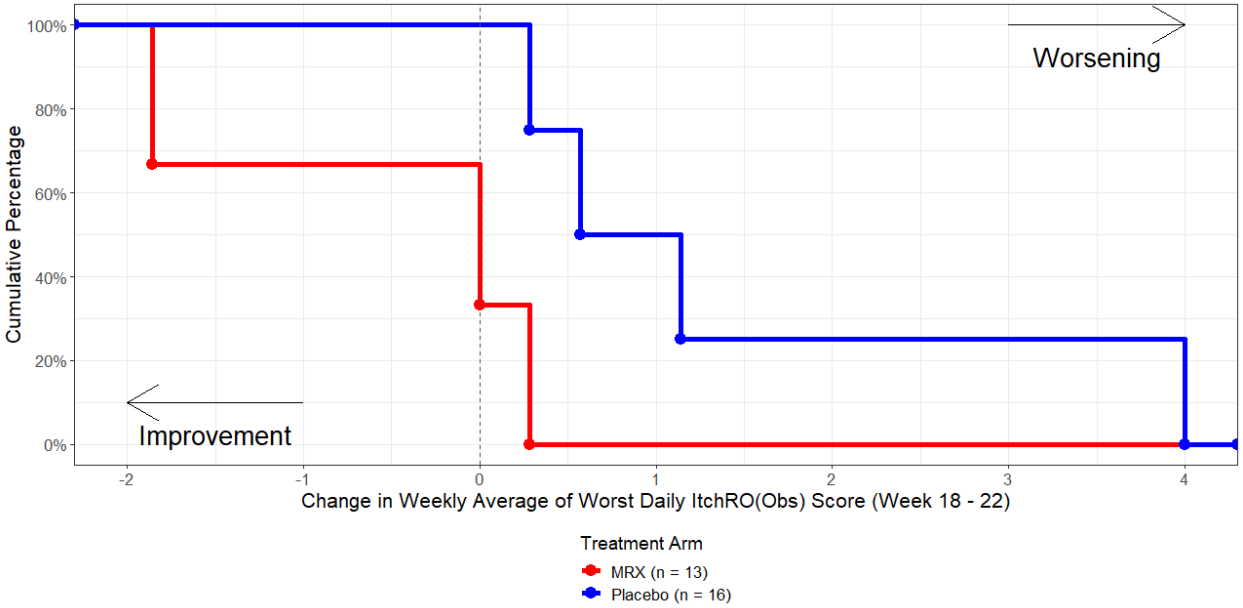
The FDA conducted post hoc analyses of empirical cumulative distribution function curves of within-patient changes in pruritus scores from Week 18 by treatment arm for the following pruritus endpoints of interest:

- Change from Week 18 (pre-randomization) to Week 22 in the weekly average of the:
  - Worst daily ItchRO(Obs) scores
  - Daily average of the morning and evening ItchRO(Obs) scores
  - Morning ItchRO(Obs) scores
  - Evening ItchRO(Obs) scores

Note that the empirical cumulative distribution function curves were created using raw change scores, consistent with the current recommendation (FDA 2019), and were based on the Applicant-submitted dataset ADQS2.xpt (Section 6.2.1.3). Figure 9 shows the cumulative percentage of patients with a given change in weekly average of worst scratching score from Week 18 to 22 by treatment arm. Approximately 54% of patients on maralixibat had worsening scores during the RWD period compared to 100% of placebo patients. Worsening of patients in the maralixibat group during the RWD period is not unexpected, because patients are transitioning from an OL treatment period, where caregivers and patients know the patient is receiving active treatment, to a randomized, double-blind, placebo-controlled period where caregivers and patients know that there is a possibility that the patient was withdrawn from active treatment. Results for the other three pruritus endpoints showed similar results (refer to Section III.16.1.4 for additional discussions and supportive analyses). Table 17 summarizes the cumulative percentage of patients experiencing worsening in pruritus during the RWD period across the four pruritus endpoints of interest.

[1] The lack of blinding to treatments can pose challenges to the interpretation of Clinical Outcome Assessment data. Specifically, knowledge of treatment use by patients, caregivers, or clinicians can lead to a systematic overestimation or underestimation of the effect of treatment.

Figure 9. eCDF curves by Treatment Arm for Change in Weekly Average of Worst Daily ItchRO(Obs) Score During the Randomized Withdrawal Period



Source: PFSS Reviewer's Figure using the Applicant-submitted dataset adqs2.xpt.
Abbreviations: eCDF, empirical cumulative distribution function; ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PFSS, Patient-Focused Statistical Support

Table 17. Percentage of Patients Reporting Worsening Pruritus During the Randomized Withdrawal Period

	Percentage of Patients Worsening[1]	
Endpoint	**MRX**	**Placebo**
Weekly average of worst daily ItchRO(Obs) scores	54%	100%
Weekly average of daily average of the morning and evening ItchRO(Obs) scores	69%	100%
Weekly average of morning ItchRO(Obs) scores	66%	100%
Weekly average of evening ItchRO(Obs) scores	54%	100%

Source: PFSS Reviewer's table using the Applicant-submitted dataset adqs2.xpt.
[1] Worsening is defined as any numerical increase in pruritus scores from Week 18.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PFSS, Patient-Focused Statistical Support

Conclusion

Given the large treatment effect observed across multiple pruritus endpoints, anchor-based analyses for the evaluation of clinically meaningful within-patient change in pruritus were not needed to interpret the treatment effect. The observed change in pruritus scores between Week 18 and Week 22 appears to represent a meaningful treatment benefit to patients and caregivers.

7. Risk and Risk Management

The overall assessment of safety of maralixibat is informed by variety of sources, including nonclinical toxicology, safety pharmacology studies, and early phase clinical studies. The safety assessment for the intended population of patients with ALGS is based primarily on Study LUM001-304. Prior to NDA submission, the clinical review team identified several potential risks based on the mechanism of action of the drug and AE reporting to the IND.

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

The overall documentation of the nonclinical program to support the safe use of maralixibat in clinical development and as an approved drug is summarized here. The nonclinical safety profile of maralixibat was evaluated in safety pharmacology studies in rats and dogs, repeat-dose oral toxicology studies in rats for up to 6 months and in dogs for up to 12 months, reproductive and developmental studies in rats and rabbits, genetic toxicology studies (Ames, in vitro chromosomal aberration, and in vivo rat micronucleus assays), and a carcinogenicity study in transgenic mice. The data from these studies are summarized below.

Safety Pharmacology

Maralixibat administered as a single oral dose (up to 150 mg/kg) or intravenous dose (up to 1.9 mg/kg) had no significant effect on neurobehavioral functions in rats, cardiovascular function in dogs, or respiratory function in guinea pigs. Maralixibat at concentrations up to 1 µmol/L did not inhibit hERG current.

General Toxicology

The most frequently observed effect in the toxicity studies was reduction in FSVs (A, D, E) in plasma and liver, which occurred in rats and in dogs. The decrease in FSVs was considered to be adverse at the highest tested doses in rats, in which deaths due to hemorrhage were observed. The bleeding-related deaths were likely a direct result of vitamin K deficiency, as reflected by prolongation of coagulation times (prothrombin time and activated partial thromboplastin time). In the 26-week rat toxicity study, maralixibat at 750 mg/kg/day in males and 2000 mg/kg/day in females caused deaths due to bleeding, which was attributed to vitamin K deficiency. Systemic exposure (area under the curve [AUC]) was not measured at the time of death. No fatal bleeding occurred after the doses were reduced to 300 mg/kg/day in males and 1500 mg/kg/day in females; however, significant prolongation of PT and APTT was observed. The reduction in FSVs is a secondary effect of maralixibat, caused by inhibition of the reabsorption of BAs, resulting in BA depletion and malabsorption of FSVs. The reductions in FSVs occurred at very high AUC multiples relative to the human AUC (35 to 2124-fold the human AUC of 0.6 and 0.161 ng•h/mL at 50 and 20 mg/day, respectively; the human AUC range of 20 to 50 mg/day approximates the human AUC at the maximum recommended dose, 28.5 mg/day, for which no AUC is available). However, none of the toxicity studies established a no-effect dose for reductions in the serum or liver levels of fat-soluble vitamins. Therefore, the animal data could be relevant to the risk in humans, suggesting a need for monitoring of the levels of FSVs in patients.

Non-AEs that occurred only in rats included an increased incidence and severity of mucus depletion of goblet cells and edema in the mucosal/submucosal lamina propria of the cecum, colon, and rectum, and nonsuppurative inflammation of the mucosa/submucosa of the cecum. These effects were observed in all toxicology studies in rats at all tested doses, and were likely related to the increased concentration of BAs in the lumen of the large intestine (pharmacologic effect of the test article).

In a 1-year toxicity study in dogs, the no observable adverse effect level in both sexes was 5 mg/kg/day with an area under the concentration-time curve from time 0 to 24 h (AUC_{0-24h}) of 57.7 ng•h/mL on Day 358 (96 to 358-fold the human AUC of 0.6 and 0.161 ng•h/mL at 50 and 20 mg/day, respectively). The no observable adverse effect level designation in males is primarily based on the nonreversible changes in stomach (e.g., increased cytoplasmic vacuolation of the pyloric mucosa), with additional consideration of the small decreases in bodyweight (6.9 to 7.8%) and weight gain (18 to 26%) at 20 and 100 mg/kg/day. The no observable adverse effect level designation in females is primarily based on the decreases in bodyweight (12.2 to 14.9%) and body-weight gain (47 to 54%) at 20 and 100 mg/kg/day.

Reproductive and Developmental Toxicology and Juvenile Animal Toxicology

Reproductive and developmental toxicology studies included a fertility study in male and female rats, embryo-fetal development studies in rats and rabbits, and a pre- and post-natal development study in rats. Two juvenile animal toxicity studies were performed in rats. No adverse findings were observed in the reproductive and developmental toxicology studies. In a juvenile rat study, maralixibat at 250 mg/kg/day produced significant increases in bone mineral density in males and a significant increase in distal femur total slice area, associated with significant increases in bone mineral content and bone mineral density in females. However, the bone effects occurred at 250 mg/kg/day with an AUC_{0-24h} of 471 ng•h/mL, which is 785 to 2925-fold the human AUC of 0.6 and 0.161 ng•h/mL at 50 and 20 mg/day, respectively. Because these effects occurred at extremely high multiples of the human AUC, they are not clinically relevant.

Genetic Toxicology

Maralixibat was not mutagenic or clastogenic in the Ames test, the in vitro chromosomal aberration test using Chinese hamster ovary cells, or the in vivo rat micronucleus assay.

Carcinogenicity

In a 6-month carcinogenicity study in transgenic mice (RasH2) study, maralixibat was not tumorigenic at oral doses up to 25 mg/kg/day in males and 75 mg/kg/day in females. Systemic exposure to maralixibat (AUC_{0-24h}) at the maximum dose studied was approximately 1490 ng•h/mL in males and 400 ng•h/mL in females.

A 2-year rat carcinogenicity study is currently in progress. The incomplete status of the carcinogenicity testing program is in accordance with the FDA's prior agreement with the Applicant. Carcinogenicity studies in two rodent species are routinely expected to support the approval of drugs for which the duration of use is expected to exceed 6 months. Therefore, submission of the 2-year rat carcinogenicity study should be a Postmarketing Requirement. The study results will be included in subsection 13.1 of the labeling, in addition to the results of the completed carcinogenicity study in Tg.RasH2 mice.

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

BA transporters maintain the enterohepatic circulation, which is crucial for BA homeostasis. The ASBT/IBAT is the major transporter that reclaims intestinal BAs. Mutations of the IBAT result in BA malabsorption diarrhea, and reduced plasma low-density lipoprotein cholesterol (Oelkers

et al. 1997). Similarly, inhibition by pharmacological agents, such as maralixibat, results in AEs of malabsorptive diarrhea, changes in BA homeostasis, and fatty-acid malabsorption (Huff et al. 2002; West et al. 2002). The sequelae of changes in BA and fatty acid homeostasis are a rebound in cholesterol biosynthesis (Huff et al. 2002), FSV deficiencies and complications thereof, such as fractures and increased risk of bleeding (Hofmann 1999). The subjects with TEAEs related to IBAT inhibition are discussed in Section 7.6. Odevixibat, a recently approved (July 2021) IBAT inhibitor, had preapproval safety data that appear similar to the preapproval safety data in this NDA. Postmarketing safety data for the IBAT inhibitor odevixibat have not been accumulated, and therefore the risks or safety concerns are described in the preapproval safety data for this NDA.

7.3. Potential Safety Concerns Identified Through Postmarket Experience

Not applicable. Maralixibat is not yet commercially available in any country.

7.4. FDA Approach to the Safety Review

Clinical study data were analyzed using JMP and JMP Clinical software. Clinical data scientists (CDSs) from the Division of Biomedical Informatics generated the safety tables and figures. These include safety tables and figures for patient disposition, exposure data, AEs, and SAEs. The CDSs also generated tables for drug-induced liver injury assessments. The Division of Biomedical Informatics, Research & Biomarker Development also provided technical support for data analysis using JMP and JMP Clinical software.

Basis for Safety Evaluation

Primary Safety Database

Data from a single study, LUM001-304, a long-term, OL study with a double-blind, placebo-controlled, RWD period of 4-week duration. The study evaluated the effects of maralixibat 400 mcg/kg on cholestatic pruritus in children with ALGS. The clinical study consisted of an 18-week OL run-in period, 4-week RWD phase, 26-week stable dosing at doses up to 400 mcg/kg/day (ARW period), and optional long-term treatment period (LTE phase). During the LTE phase, the maralixibat dose could be increased to a maximum of 800 µg/kg/day (maralixibat at 400 mcg/kg twice daily), depending on sBA level, ItchRO(Obs) score, and safety assessments. At the time of data-cut off (December 1, 2019), the longest follow up time point was Week 264. A summary of the LUM001-304 design can be found in Section 6.2.1.

Supportive Safety Database

Data from two randomized, placebo-controlled, Phase 2 studies of 13-week duration (LUM001-301 and LUM001-302) with optional LTE studies (LUM001-305 and LUM001-303, respectively). Lower doses of maralixibat were administered in these studies (LUM001-301 (≤140 mcg/kg/day), LUM001-302 (≤280 mcg/kg/day), LUM001-305 (≤280 mcg/kg/day), and LUM001-303 (maralixibat at ≤140 mcg/kg/day) than the proposed dose of 400 mcg/kg daily used in the pivotal study, LUM001-304. For safety analyses, the reviewer used the pooled safety dataset from all five studies (LUM001-301, -302, -303, -304, and -305).

No major data quality or integrity issues were identified that would preclude the clinical review team from performing a safety review for this NDA. Data quality and integrity were verified by the FDA's CDS team as well as the clinical reviewer. There were no major identified issues with respect to recording, coding, and categorizing AEs. However, several preferred terms (PTs) were recoded to analyze adverse events of special interest (AESIs), as shown in Table 18.

These recoded terms were used by the CDS team for analyzing the safety data, in addition to the analyses performed by the Applicant coding and categorization of AEs.

Table 18. Reviewer's Recoding from Adverse Event Terms

Medical Officer Recording	Adverse Event Terms
Abdominal pain	Abdominal pain, upper abdominal pain, lower abdominal pain
Diarrhea	Diarrhea, loose stools, increase bowel movements, increased stools
Fat-soluble vitamin deficiency	Fat-soluble vitamin deficiency, vitamin A abnormal, vitamin D deficiency, vitamin D decreased, vitamin E decreased, vitamin E deficiency, vitamin K deficiency
Aminotransferase increased	ALT and AST increased
Bilirubin increased	Blood bilirubin increased, increased bilirubin
Fractures	Fractures, clavicular fractures, forearm fracture, hand fracture, humerus fracture, pathological fracture, rib fracture, tibia fracture
Hemorrhage	Hemorrhage, catheter site hemorrhage, ear hemorrhage, epistaxis, gastrointestinal hemorrhage, lip hemorrhage, subdural hemorrhage, mouth hemorrhage, post procedural hemorrhage, procedural hemorrhage, wound hemorrhage
Decreased platelets	Decreased platelets, thrombocytopenia
Nasopharyngitis	Nasopharyngitis, pharyngitis
Infections	Infections, lower respiratory tract infection, upper respiratory tract infection, viral infection, viral pharyngitis, rotavirus infection, gastroenteritis, campylobacter gastroenteritis, tonsillitis
Fever	Fever, pyrexia
INR increased	International normalized ratio increased, INR increased, increased INR, abnormal INR
Thrombocytopenia	Thrombocytopenia, decreased platelets.
Growth retardation	Growth retardation, weight decreased

Source: Adapted from the clinical data scientist's Word file: Study LUM001-304 and ISS.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio

7.4.1. Safety Analysis Plan and Definitions

The prespecified safety analysis plan and definitions were reviewed during protocol development and were acceptable to the clinical review team. Use of descriptive statistics was predefined in the protocol for summarizing the safety outcomes. The review team agreed with the proposed approach. All AEs were classified by primary System Organ Class and PT according to the Medical Dictionary for Regulatory Activities version 23.0.

An AE is defined as any untoward medical occurrence in an enrolled patient irrespective of a causal relationship with study drug.

TEAEs were defined as an AE (PT) that occurs during the treatment period plus 14 days with a start date on or after the first dose date of study drug, or a start date before the date of the first dose date of study drug, but worsened in severity on or after the date of the first dose date of study drug. In cases where study drug was interrupted or withdrawn for >14 days, then definition of a TEAE will take into consideration the date of the last dose before study drug interruption

and the actual last dose. AEs that commenced >14 days after the last dose before study drug interruption, and ended before the drug was readministered, will not be considered TEAEs.

AESIs were classified based on preclinical and clinical experience. The following AESIs were prespecified in the protocol:

- Diarrhea.
- FSV deficiency.
- Elevated transaminases.
- Elevated bilirubin.

SAEs were protocol-defined to match with regulation (21 CFR 312.32(a)) as any untoward medical occurrence that, at any dose:

- Results in an outcome of death.
- Is life threatening (defined as, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred).
- Results in persistent or significant disability/incapacity (defined as disruption of the ability to conduct normal life functions).
- Requires or prolongs hospitalization.
- Results in a congenital anomaly or birth defect.
- Is an important medical event (defined as representing a significant hazard, or requires medical and surgical intervention to prevent any of the outcomes listed above).

Severity assessments were classified as follows:

- Mild (awareness of sign and symptom, but easily tolerated).
- Moderate (discomfort sufficient to interfere with normal activities).
- Severe (incapacitating, with inability to perform normal activities).

A severe AE was considered an SAE only if it met the criteria above for an SAE.

7.5. Adequacy of Clinical Safety Database

The size of the safety population was 31 for LUM001-304 and 86 for the drug development program, which was adequate to conduct a safety review of maralixibat for cholestatic pruritus in ALGS. LUM001-304 represents the only adequate and well-controlled clinical study of maralixibat for cholestatic pruritus in ALGS and was conducted in Europe and Australia. The two supportive studies (LUM001-301 and -302 with LTEs, -305 and -303, respectively) were conducted in the United States and Canada. The disease presentation is similar worldwide. ALGS is a rare disease with an approximate prevalence in the United States of 1 in 30,000 to 1 in 70,000 (Danks et al. 1977; Leonard et al. 2014).

The safety population was defined as subjects who received at least one dose of study drug. One hundred and nineteen subjects were screened of which there were 31 screen failures and 88 who were enrolled. Eighty-six of the 88 who enrolled received at least one dose of MRX. However, two subjects who enrolled in LUM001-301 (and received at least one dose of MRX) did not roll-over into the long-term extension studies. These two subjects were included in the ISS summary tables for the two supportive studies (301/302) but not in the open label/extension safety population. Therefore, the summary tables of the safety review consist of 84 subjects. Narratives

of safety events of the two subjects (neither subject had a SAE) who did not roll over into open-label extension studies are described separately. Safety data for all 86 subjects are reported in the label.

The mean (SD) durations of exposure to maralixibat in LUM001-304 during the OL-maralixibat (31 subjects), RWD (29 subjects), ARW-maralixibat (29 subjects), LTE-maralixibat-QD (23 subjects), and LTE-maralixibat-BID (15 subjects) periods were 121.4 (22.5), 29.5 (3.5), 182.1 (15.4), 404.5 (211.7), and 702 (215.8) days, respectively (Table 19).

Table 19. Baseline Demographic and Clinical Characteristics, Safety Population, LUM001-304

Characteristic	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)
Sex, n (%)					
Female	12 (38.7)	4 (30.8)	6 (37.5)	10 (34.5)	9 (39.1)
Male	19 (61.3)	9 (69.2)	10 (62.5)	19 (65.5)	14 (60.9)
Age, years					
Mean (SD)	5.4 (4.2)	5.4 (5)	5.8 (3.8)	5.6 (4.3)	6.1 (4.3)
Median (min, max)	4.5 (1, 15)	4 (1, 15)	5 (1, 14)	5 (1, 15)	5 (1, 15)
Age group, years, n (%)					
<2 years	6 (19.4)	3 (23.1)	2 (12.5)	5 (17.2)	4 (17.4)
2 to 4 years	9 (29.0)	5 (38.5)	3 (18.8)	8 (27.6)	4 (17.4)
5 to 8 years	9 (29.0)	2 (15.4)	7 (43.8)	9 (31.0)	9 (39.1)
9 to 12 years	4 (12.9)	1 (7.7)	3 (18.8)	4 (13.8)	3 (13.0)
13 to 18 years	3 (9.7)	2 (15.4)	1 (6.2)	3 (10.3)	3 (13.0)
Ethnicity, n (%)*					
Value missing	31 (100)	13 (100)	16 (100)	29 (100)	23 (100)
Race, n (%)*					
Value missing	31 (100)	13 (100)	16 (100)	29 (100)	23 (100)
Country of participation, n (%)					
Australia	9 (29.0)	5 (38.5)	4 (25.0)	9 (31.0)	9 (39.1)
Belgium	5 (16.1)	1 (7.7)	2 (12.5)	3 (10.3)	3 (13.0)
Spain	3 (9.7)	2 (15.4)	1 (6.2)	3 (10.3)	1 (4.3)
France	9 (29.0)	3 (23.1)	6 (37.5)	9 (31.0)	6 (26.1)
Great Britain	3 (9.7)	2 (15.4)	1 (6.2)	3 (10.3)	3 (13.0)
Poland	2 (6.5)	0 (0)	2 (12.5)	2 (6.9)	1 (4.3)

Source: Clinical data scientist's Table 2; February 16, 2021.
*Ethnicity and race were not captured in LUM001-304, see the discussion below.
Abbreviations: ARW, after randomized withdrawal; LTE, long-term extension; max, maximum; min, minimum; MRX, maralixibat; OL, open-label; PLB, placebo; RWD, randomized withdrawal

Enrolled subjects: 31 (30 planned) in nine centers (six countries). Two subjects withdrew during the OL period and 29 were randomized to the 4-week placebo-controlled period. The safety population consisted of 31 subjects who participated in LUM001-304.

Characteristics: Two of the thirty-one enrolled subjects dropped out due to AEs during the OL portion of the study. Thirteen of the twenty-nine subjects who completed the RWD portion of LUM001-304 were assigned to maralixibat and 16 to placebo. The sex distribution in each of the two arms was similar to the overall study population, with males comprising>60% of the population in each arm. The age distribution and mean (SD) age were similar in the RWD-maralixibat (5.4 [5] years) and RWD-placebo (PLB) (5.8 [3.8] years) arms. No subjects were enrolled from the United States. Disease severity was defined by prespecified laboratory, genetic, and clinical criteria, as outlined in the inclusion and exclusion criteria (Section 6.2.1.2).

Substantial differences in disease characteristics between countries are not expected, although there may be some variability in the management of patients with ALGS. See Table 20.

The Applicant's submission did not contain race or ethnicity data for patients in Study LUM001-304. In response to an IR from the FDA on March 2, 2021, the Applicant provided the following explanation for not collecting race and ethnicity data in Study LUM001-304:

> *"collection of race and ethnicity in France was prohibited according to data protection regulation Loi n° 78-17 du 6 janvier 1978 relative à l'informatique, aux fichiers et aux libertés, Article 6… In addition, at that time, a Canadian site ethics committee requested justification to collect race. Therefore, the study sponsor at the time decided to refrain from collection of race information in Study LUM001-304."*

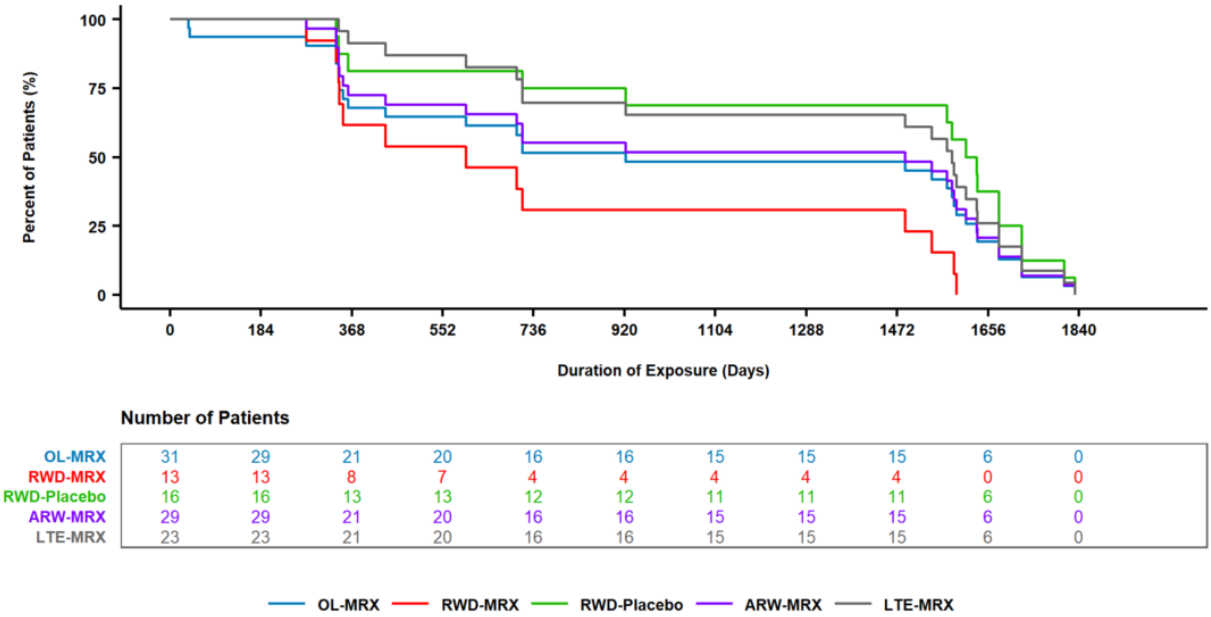
Table 20. Duration of Exposure, Safety Population, Study LUM001-304

Variable	OL-MRX N=31 n (%)	RWD N=29 n (%)	ARW-MRX N=29 n (%)	LTE-MRX-QD N=23 n (%)	LTE-MRX-BID N=15 n (%)
Duration of exposure, days					
Mean (SD)	121.4 (22.5)	29.5 (3.5)	182.1 (15.4)	404.5 (211.7)	702.4 (215.8)
Median (Q1, Q3)	126 (125, 131)	28 (28, 30)	185 (175, 189)	412 (353, 464.5)	752 (720, 819)
Minimum, maximum	37, 135	25, 40	116, 203	5, 979	27, 845
Total exposure (person years)	10.3	2.3	14.5	25.5	28.8
Patients treated, by duration, n (%)					
<100 days	29 (93.5)	0 (0)	29 (100)	1 (4.3)	0 (0)
≥100 to <300 days	2 (6.5)	29 (100)	0 (0)	3 (13)	1 (6.7)
≥300 to <600 days	0 (0)	0 (0)	0 (0)	17 (73.9)	1 (6.7)
≥600 to <1000 days	0 (0)	0 (0)	0 (0)	2 (8.7)	13 (86.7)

Source: Clinical data scientist's Table 5; February 16, 2021.
Abbreviations: ARW, after randomized withdrawal; BID, twice a day; LTE, long-term extension; MRX, maralix bat; OL, open-label; QD, one a day; RWD, randomized withdrawal

The mean (SD) exposure duration for RWD in LUM001-304 was 29.5 (3.5) days (Figure 10).

Figure 10. Duration of Exposure, Safety Population, Study LUM001-304



Source: Clinical data scientist's Figure 1; February 16, 2021.
Abbreviations: ARW, after randomized withdrawal; LTE, long-term extension; MRX, maralixibat; OL, open-label; RWD, randomized withdrawal

Integrated Summary of Safety

The safety population for the ISS had similar demographics to the population in Study LUM001-304. Mean age (SD) was 6.1 (4.5) years

The ISS analyses were also used to assess safety, due to the longer duration of exposure which allowed for identification of more safety signals as compared with a 4-week RWD period. Mean (SD) duration of exposure to maralixibat was 913.1 (532) days in the ISS (Table 21) as compared to a mean (SD) of 29.5 (3.5) days in the RWD of LUM001-304.

Information regarding race and ethnicity was available for subjects from the two supportive studies (LUM001-301 and -302 and open-label extension studies LUM001-305 and LUM001-303, respectively) conducted in the US, Canada, and the UK. Most were white [(44/55 ((80%)] and slightly more than half were male [30/55 (55%)].

The most common SAEs in the safety population were infections (8/84, 9.5%), GI disorders (7/84, 8.3%), and injuries and procedural complications (6/84, 7.1%). Infections (7/36 [19.4%] in the >280 mcg dose cohort and 1/10 [10%] in the ≤140 mcg dose cohort) were primarily bacterial or viral gastroenteritis (4/36, 10%). GI Disorders consisted of GI bleeding (3/84, 3.5%) in the ≤140 mcg dose cohort only, vomiting (2/84, 2.4%) at equal rates in the 280 mcg (1/38, 2.6%) cohort and >280 mcg dose cohort (1/36, 2.7%), abdominal pain (1/84, 1.2%), and diarrhea (1/84, 1.2%).

Evaluation of SAEs by PT revealed that hemorrhage (7/84, 2.4%) was the most frequent SAE, occurring most commonly in 2/10 (20%) in the ≤140 mcg dose cohort followed by the >280 mcg dose cohort (4/36, 11.1%), and the 280 mcg dose cohort (1/38, 2.6%). Vomiting (3/84, 3.6%) occurred at identical rates in the three dose cohorts; pyrexia (2/84, 2.4%) and anemia occurred at the same frequency, but pyrexia occurred only in the >280 mcg dose cohort and anemia (2/84, 2.4%) at equal rates in the ≤140 mcg and >280 mcg dose cohorts.

Table 21. Duration of Exposure, Safety Population, Integrated Summary of Safety

Variable	≤140 mcg N=10 n (%)	280 mcg N=38 n (%)	>280 mcg N=36 n (%)	Overall Maralixibat N=84 n (%)
Duration of exposure, days				
Mean (SD)	795 (567.9)	844.5 (446.5)	1018.3 (597.4)	913.1 (532)
Median (Q1, Q3)	1008.5 (192.5, 1304)	836.5 (504, 1295.2)	1396.5 (330.2, 1540)	913 (406.8, 1350)
Minimum, maximum	25, 1336	58, 1612	37, 1731	25, 1731
Total exposure (person-years)	22	88	100	210
Patients treated by duration, n (%)				
<13 weeks	2 (20.0)	1 (2.6)	2 (5.6)	5 (6.0)
≥13 to <23 weeks	1 (10.0)	0	0	1 (1.2)
≥23 to <78 weeks	1 (10.0)	15 (39.5)	9 (25.0)	25 (29.8)
≥78 to <104 weeks	0	1 (2.6)	5 (13.9)	6 (7.1)
≥104 to <156 weeks	1 (10.0)	5 (13.2)	1 (2.8)	7 (8.3)
≥156 to <208 weeks	5 (50.0)	15 (39.5)	1 (2.8)	21 (25.0)
≥208 weeks	0	1 (2.6)	18 (50.0)	19 (22.6)

Source: Clinical data scientist's Table 5; March 17, 2021; adex.xpt; software, R.
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with given treatment duration; SD, standard deviation; Q1, first quartile; Q3, third quartile

7.6. Safety Findings and Concerns Based on Review of Clinical Safety Database

7.6.1. Safety Findings and Concerns, Study LUM001-304

7.6.1.1. Overall TEAE Summary, Study LUM001-304

When subjects treated with maralixibat and placebo were compared in the RWD period, no difference in overall SAEs was found. Also, there was no difference in AEs leading to discontinuation of study drug for maralixibat (0) versus placebo (0) or AEs leading to dose modification for maralixibat (0) or placebo (1/16, 6.2%). In addition, the difference in AEs between maralixibat and placebo was not substantial, as shown by a risk difference (RD) and 95% CI of -21.2 (-55.6, 13.3). In the subsequent tables, note that the risk difference column shows the difference (95% confidence interval) between RWD-MRX and RWD-PLB.

The most common TEAEs were abdominal pain, diarrhea, nasopharyngitis, and vomiting during the OL period (13/31 [41.9%] each for abdominal pain and diarrhea; 4/31 [12.9%] for nasopharyngitis; and 11/31 [35.5%] for vomiting). During the LTE period, the TEAEs were abdominal pain (12/23, 52.2%), diarrhea (7/23, 30.4%), nasopharyngitis (9/23, 39.1%), and vomiting (8/23, 34.8%). Lastly, the TEAEs in the ARW period were abdominal pain (6/23, 20.7%), diarrhea (5/23, 17.2%), nasopharyngitis (8/23, 27.6%), and vomiting (3/23, 10.3%). More cases of increased transaminases occurred during the LTE period. See Table 22.

Table 22. Overview of Treatment-Emergent Adverse Events, Controlled Study LUM001-304 Safety Population

Preferred Term	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RDW-PLB N=1 n (%)	ARW-MRX N=23 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Abdominal pain	13 (41.9)	1 (7.7)	1 (6.2)	6 (20.7)	12 (52.2)	1.5 (-17.3, 20.2)
Diarrhea	13 (41.9)	1 (7.7)	1 (6.2)	5 (17.2)	7 (30.4)	1.5 (-17.3, 20.2))
Vomiting	11 (35.5)	1 (7.7)	1 (6.2)	3 (10.3)	8 (34.8)	1.5 (17.3, 20.2)
Nasopharyngitis	4 (12.9)	1 (7.7)	1 (6.2)	8 (27.6)	9 (39.1)	1.5 (-17.3, 20.2)
Pyrexia	6 (19.4)	0	2 (12.5)	7 (24.1)	10 (43.5)	-12.5 (-28.7, 3.7)
Pruritus	3 (9.7)	1 (7.7)	5 (31.2)	2 (6.9)	0	-23.5 (-50.5, 3.4)
ALT	0	0	0	0	4 (17.4)	0
AST	0	0	0	0	2 (8.7)	0 (0)
INR increase	1 (3.2)	0	0	0	1 (4.3)	0

Source: Clinical data scientist's Table 13; February 16, 2021.
Abbreviations: ALT, alanine aminotransferase; ARW, after randomized withdrawal; AST, aspartate aminotransferase; CI, confidence interval; INR, international normalized ratio; LTE, long-term extension; MRX, maralixibat; OL, open-label; PLB, placebo; RDW, randomized withdrawal

Adverse Events for LUM001-304

No deaths were reported during Study LUM001-304 or in the safety population. See liver transplant from LUM001-304 in Supplement 17, Liver Transplantation.

7.6.1.2. Serious Adverse Events, Pivotal Study, LUM001-304

When compared across treatment groups in the pivotal study, no RDs were discovered in the common GI AESIs of abdominal pain, diarrhea, or vomiting. In addition, one subject (1/16,

6.2%) in the placebo arm experienced pyrexia, splenic rupture, and hemorrhagic shock events compared to 0 patients in the maralixibat arm (RD [95% CI] -6.2 [-18.1, 5.6]).

Serious Adverse Events

The Applicant reported that 14 subjects experienced 33 SAEs, none of which attributed to the study drug. Our review of these findings coincided with the Applicant's reports. See Table 23.

Table 23. Serious Adverse Events by System Organ Class and Preferred Term, Safety Population, Study LUM001-304

System Organ Class Preferred Term	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Blood and lymphatic system disorders	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Aplasia pure red cell	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Cardiac disorders	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Cardiac dysfunction	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Gastrointestinal disorders	1 (3.2)	0 (0)	0 (0)	2 (6.9)	0 (0)	0 (0, 0)
Abdominal pain	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Diarrhea	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Vomiting	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
General disorders and administration site conditions	0 (0)	0 (0)	1 (6.2)	0 (0)	2 (8.7)	-6.2 (-18.1, 5.6)
Pyrexia	0 (0)	0 (0)	1 (6.2)	0 (0)	1 (4.3)	-6.2 (-18.1, 5.6)
Infections and infestations	2 (6.5)	1 (7.7)	0 (0)	2 (6.9)	2 (8.7)	7.7 (-6.8, 22.2)
Viral infection	0 (0)	1 (7.7)	0 (0)	0 (0)	0 (0)	7.7 (-6.8, 22.2)
Campylobacter gastroenteritis	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Epstein-Barr virus infection	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Gastroenteritis	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Rotavirus infection	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Tonsillitis	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Viral pharyngitis	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Injury, poisoning and procedural complications	1 (3.2)	0 (0)	1 (6.2)	1 (3.4)	2 (8.7)	-6.2 (-18.1, 5.6)
Extradural hematoma	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Forearm fracture	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Subdural hemorrhage	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Toxicity to various agents	0 (0)	0 (0)	0 (0)	1 (3.4)	1 (4.3)	0 (0, 0)
Splenic rupture	0 (0)	0 (0)	1 (6.2)	0 (0)	0 (0)	-6.2 (-18.1, 5.6)
Investigations	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Blood bilirubin increased	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Neoplasms benign, malignant, and unspecified (cysts and polyps)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Marrow hyperplasia	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Nervous system disorders	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Seizure	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Renal and urinary disorders	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Acute kidney injury	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)

System Organ Class Preferred Term	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Vascular disorders	0 (0)	0 (0)	1 (6.2)	1 (3.4)	0 (0)	-6.2 (-18.1, 5.6)
Shock hemorrhagic	0 (0)	0 (0)	1 (6.2)	0 (0)	0 (0)	-6.2 (-18.1, 5.6)

Source: Clinical data scientist's Table 9; February 16, 2021.
Abbreviations: ARW, after randomized withdrawal; CI, confidence interval; LTE, long-term extension; MRX, maralixibat; N, number of patients in treatment arm; n, number of patients with adverse event; OL, open-label; PLB, placebo; RWD, randomized withdrawal; SOC, system organ class

Upon further assessment using FDA Medical Query (FMQ) terms, the RDs for GI disorders and pyrexia were unchanged. Most SAEs were pyrexia, viral infections, and hemorrhagic shock. The small size of the database limited RD precision. Note that the RD for hemorrhage narrowed by FMQ was the same as hemorrhagic shock and splenic rupture, see Table 24.

The SAE of hemorrhagic shock was unrelated to maralixibat, it occurred in Subject (b) (6) who suffered a fall from a horse resulting in splenic rupture and hemorrhagic shock.

Table 24. Serious Adverse Events by System Organ Class and FDA Medical Query (Narrow), Safety Population, Study LUM001-304

System Organ Class FMQ (Narrow)	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Blood and lymphatic system disorders						
Anemia	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0, 0)
Cardiac disorders						
Systemic hypertension	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Gastrointestinal disorders						
Abdominal pain	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Diarrhea	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Vomiting	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
General disorders and administration site conditions						
Pyrexia	0 (0)	0 (0)	1 (6.2)	0 (0)	1 (4.3)	-6.2 (-18.1, 5.6)
Infections and infestations						
Nasopharyngitis	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Viral infection	0 (0)	1 (7.7)	0 (0)	0 (0)	0 (0)	7.7 (-6.8, 22.2)
Injury, poisoning, and procedural complications	1 (3.2)	0 (0)	1 (6.2)	1 (3.4)	2 (8.7)	-6.2 (-18.1, 5.6)

System Organ Class FMQ (Narrow)	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Nervous system disorders						
Seizure	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Renal/urinary disorders						
Acute kidney injury	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Vascular disorders	0 (0)	0 (0)	1 (6.2)	1 (3.4)	0 (0)	-6.2 (-18.1, 5.6)
Shock, hemorrhagic	0 (0)	0 (0)	1 (6.2)	0 (0)	0 (0)	-6.2 (-18.1, 5.6)
Hemorrhage	1 (3.2)	0 (0)	1 (6.2)	0 (0)	0 (0)	-6.2 (-18.1, 5.6)

Source: Clinical data scientist's Table 25; February 16, 2021.
Abbreviations: ARW, after randomized withdrawal; CI, confidence interval; LTE, long-term extension; MRX, maralixibat; N, number of patients in treatment arm; n, number of patients with adverse event; OL, open-label; PLB, placebo; RWD, randomized withdrawal; SOC, system organ class

7.6.1.3. Dropouts and/or Discontinuations Due to Adverse Events, Study LUM001-304

No differences were observed in the RWD period that led to discontinuation of maralixibat. However, two cases of hepatic injury in the LTE and one in the OL who experienced splenic rupture (due to trauma) led to discontinuation of maralixibat; and one in the ARW from acute kidney injury. See Table 25.

Table 25. Adverse Events Leading to Discontinuation by System Organ Class and FDA Medical Query (Narrow), Safety Population, Study LUM001-304

System Organ Class FMQ (Narrow)	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Hepatobiliary disorders						
Hepatic injury	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.7)	0 (0, 0)
Renal and urinary disorders						
Acute kidney injury	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Vascular disorders						
Hemorrhage	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)

Source: Table 11 of the clinical data scientist's analyses, February 6, 2021.
Abbreviations: ARW, after randomized withdrawal; CI, confidence interval; LTE, long-term extension; MRX, maralixibat; N, number of patients in treatment arm; n, number of patients with adverse event; OL, open-label; PLB, placebo; RWD, randomized withdrawal; SOC, system organ class

Upon narrowing by FMQ, AEs of nausea, abdominal pain, diarrhea, and vomiting were frequent in the RWD period and had an identical incidence (1/12, 7.7% each) in subjects treated with maralixibat and placebo (except for nausea, which was not reported in the placebo group). However, during the OL-maralixibat and LTE-maralixibat periods, the consecutive occurrence of AE was abdominal pain and diarrhea (13/31, 41.9%) each for OL-maralixibat, vomiting (11/31, 35.5%), pyrexia and nasopharyngitis (6/31, 19.4% each); headache (5/31, 16.1%), and hemorrhage (3/31, 9.7%). In comparison, during the LTE-maralixibat period the most frequent AEs were abdominal pain and nasopharyngitis (12/23, 52.2%), pyrexia (10/23, 43.2%), vomiting (8/23, 34.8%), diarrhea (7/23, 30.4%), and hemorrhage (6/23, 26.1%). Headaches (4/23, 17.4%) and nausea (2/23, 8.7%) were less frequent. See Table 26.

Table 26. Adverse Events by SOC and FMQ (Narrow), Safety Population, Study LUM001-304

FMQ	OL-MRX N=31, n (%)	RWD-MRX N=12, n (%)	RWD-PLB N=16, n (%)	ARW-MRX N=29, n (%)	LTE-MRX N=23, n (%)	Risk Difference (95% CI)
Nausea	1 (3.2)	1 (7.7)	0 (0)	1 (3.4)	2 (8.7)	7.7 (-6.8, 22.2)
Abdominal pain	13 (41.9)	1 (7.7)	1 (6.2)	6 (20.7)	12 (52.2)	1.5 (-17.3, 20.2)
Diarrhea	13 (41.9)	1 (7.7)	1 (6.2)	5 (17.2)	7 (30.4)	1.5 (-17.3, 20.2)
Vomiting	11 (35.5)	1 (7.7)	1 (6.2)	3 (10.3)	8 (34.8)	1.5 (-17.3, 20.2)
Pyrexia	6 (19.4)	0 (0)	2 (12.5)	7 (24.1)	10 (43.5)	-12.5 (-28.7, 3.7)
Nasopharyngitis	6 (19.4)	2 (15.4)	2 (12.5)	8 (27.6)	12 (52.2)	2.9 (-22.6, 28.3)
Headache	5 (16.1)	0(0)	0(0)	2 (6.9)	4 (17.4)	0 (0)
Hemorrhage	3 (9.7)	0(0)	1 (6.2)	1 (3.4)	6 (26.1)	-6.2 (-18.1, 5.6)

Source: Clinical data scientist's Table 14; February 16, 2021; adae.xpt; software, R.
Treatment-emergent adverse events were defined as AEs with a start date on or after the first dose of study drug and prior to the last dose of study drug plus 14 days.
Duration is approximately 52 weeks. Participants who completed 48 weeks of treatment and were eligible to receive further treatment could continue in an initial 52-week optional long-term follow-up treatment period, after which eligible participants could continue study treatment beyond Week 52.
Risk difference column shows the difference (95% confidence interval) between RWD-MRX and RWD-PLB.
Abbreviations: ARW, after randomized withdrawal; CI, confidence interval; FMQ, FDA MedDRA query; LTE, long-term extension; MRX, maralixibat; N, number of patients in treatment arm; n, number of patients with adverse event; OL, open-label; PLB, placebo; RWD, randomized withdrawal; SOC, system organ class

No AE was substantially associated with discontinuation of study drug during the RWD period. Three subjects discontinued maralixibat while enrolled in LUM001-304, due to abnormal liver tests: one hyperbilirubinemia, and two increased ALT. However, during the OL-maralixibat period the occurrence of staphylococcal infection in one subject and extradural and subdural hematoma in one subject led to discontinuation of study drug. See Table 27.

Table 27. Adverse Events Leading to Discontinuation by System Organ Class and Preferred Term, Safety Population, Study LUM001-304

System Organ Class Preferred Term	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Infections and infestations	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Staphylococcal infection	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Injury, poisoning and procedural complications	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Extradural hematoma	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Subdural hemorrhage	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Investigations	0 (0)	0 (0)	0 (0)	1 (3.4)	2 (8.7)	0 (0, 0)
Alanine aminotransferase increased	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.7)	0 (0, 0)
Blood bilirubin increased	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Renal and urinary disorders	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Acute kidney injury	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)

Source: Clinical data scientist's Table 12; February 16, 2021; adae.xpt; software, R.
Treatment-emergent adverse events were defined as AEs with a start date on or after the first dose of study drug and prior to the last dose of study drug plus 14 days.
Duration is approximately 52 weeks. Participants who completed 48 weeks of treatment and were eligible to receive further treatment could continue in an initial 52-week optional long-term follow-up treatment period, after which eligible participants could continue study treatment beyond Week 52.
Risk difference column shows difference (95% CI) between RWD-MRX and RWD-PLB.
Abbreviations: ARW, after randomized withdrawal; CI, confidence interval; LTE, long-term extension; MRX, maralixibat; N, number of patients in treatment arm; n, number of patients with adverse event; OL, open-label; PLB, placebo; RWD, randomized withdrawal; SOC, system organ class

7.6.1.4. Treatment-Emergent Adverse Events, Study LUM001-304

An imbalance in the overall distribution of TEAEs in RDW-maralixibat (7/13 [53.8%]) compared with RDW-PLB (12/16 [75%]) was observed. However, TEAEs of abdominal pain, diarrhea, nasopharyngitis, and vomiting were most common and evenly distributed in both treatment arms of the RWD of the pivotal study, see Table 28.

Table 28. Common Adverse Events, Safety Population, Pooled Analysis

Preferred Term (PT)	OL-MRX N=31 n (%)	RWD-MRX N=13 n(%)	RDW-PLB N=16 n(%)	ARW-MRX N=23 n (%)	LTE-MRX N=23 n(%)	Risk Difference (95% CI)
Any adverse event	30 (96.8)	7 (53.8)	12 (75.0)	25 (86.2)	23 (100)	-21.2 (-55.6, 13.3)
Abdominal pain	13 (41.9)	1 (7.7)	1 (6.2)	6 (20.7)	12 (52.2)	1.5 (-17.3, 20.2)
Diarrhea	13 (41.9)	1 (7.7)	1 (6.2)	5 (17.2)	7 (30.4)	1.5 (-17.3, 20.2))
Vomiting	11 (35.5)	1 (7.7)	1 (6.2)	3 (10.3)	8 (34.8)	1.5 (17.3, 20.2)
Nasopharyngitis	4 (12.9)	1 (7.7)	1 (6.2)	8 (27.6)	9 (39.1)	1.5 (-17.3, 20.2)
Pyrexia	6 (19.4)	0	2 (12.5)	7 (24.1)	10 (43.5)	-12.5 (-28.7, 3.7)
Pruritus	3 (9.7)	1 (7.7)	5 (31.2)	2 (6.9)	0	-23.5 (-50.5, 3.4)
ALT	0	0	0	0	4 (17.4)	0
AST	0	0	0	0	2 (8.7)	0 (0)
INR increase	1 (3.2)	0	0	0	1 (4.3)	0

Source: Clinical data scientist's Table 13; February 16, 2021.
Abbreviations: ALT, alanine aminotransferase; ARW, after randomized withdrawal; AST, aspartate aminotransferase; CI, confidence interval; INR, international normalized ratio; LTE, long-term extension; MRX, maralixibat; N, number of patients in treatment arm; n, number of patients with adverse event; OL, open-label; PLB, placebo; RWD, randomized withdrawal

Adverse Events of Special Interest

No AESI occurred in RWD-maralixibat and RDW-PLB of LUM001-304 (Table 28).

7.6.1.5. Laboratory Findings, Study LUM001-304

Chemistry

In comparison to RWD-PLB, changes from normal range of sodium (2/13 [15.4%] <132 mEq/L and 0 >144 mEq/L) and potassium (0 <3.6 mEq/L [0.0%] and 1/13 [7.7%] >5.5 mEq/L) levels were seen in the RWD. However, no meaningful differences in laboratory deviations were observed between RWD-maralixibat and RWD-PLB. See Table 29.

Table 29. Patients With One or More Chemistry Analyte Values Outside Specified Levels, Safety Population, Study LUM001-304

Laboratory Parameter	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Sodium, low (mEq/L)						
Level 1 (<134)	5 (16.1)	3 (23.1)	2 (12.5)	5 (17.2)	0 (0)	10.58 (-17.48, 38.63)
Level 2 (<132)	5 (16.1)	2 (15.4)	2 (12.5)	3 (10.3)	0 (0)	2.88 (-22.56, 28.33)
Level 3 (<125)	2 (6.5)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Sodium, high (mEq/L)						
Level 1 (>144)	0 (0)	0 (0)	0 (0)	2 (6.9)	0 (0)	0 (0, 0)
Level 2 (>150)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Level 3 (>155)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)

Laboratory Parameter	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Potassium, low (mEq/L)						0 (0)
Level 1 (<3.6)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Level 2 (<3.4)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Level 3 (<3)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Potassium, high (mEq/L)						0 (0)
Level 1 (>5.5)	1 (3.2)	1 (7.7)	0 (0)	4 (13.8)	0 (0)	7.69 (-6.79, 22.18)
Level 2 (>6)	1 (3.2)	1 (7.7)	0 (0)	2 (6.9)	0 (0)	7.69 (-6.79, 22.18)
Level 3 (>6.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Chloride, low (mEq/L)						0 (0)
Level 1 (<95)	6 (19.4)	4 (30.8)	1 (6.2)	6 (20.7)	0 (0)	24.52 (-3.23, 52.27)
Level 2 (<88)	2 (6.5)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Level 3 (<80)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Chloride, high (mEq/L)						0 (0)
Level 1 (>108)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Level 2 (>112)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Level 3 (>115)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Bicarbonate, low (mEq/L)						0 (0)
Level 1 (<20)	26 (83.9)	8 (61.5)	9 (56.2)	24 (82.8)	0 (0)	5.29 (-30.63, 41.21)
Level 2 (<18)	13 (41.9)	3 (23.1)	3 (18.8)	15 (51.7)	0 (0)	4.33 (-25.51, 34.17)
Level 3 (<15)	5 (16.1)	1 (7.7)	3 (18.8)	7 (24.1)	0 (0)	-11.06 (-35.05, 12.93)
Bicarbonate, high (mEq/L)						0 (0)
Level 1 (NA)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Level 2 (NA)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Level 3 (>30)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Glucose, low (mg/dL)						0 (0)
Level 1 (<70)	9 (29.0)	2 (15.4)	2 (12.5)	11 (37.9)	0 (0)	2.88 (-22.56, 28.33)
Level 2 (<54)	0 (0)	0 (0)	0 (0)	3 (10.3)	0 (0)	0 (0, 0)
Level 3 (<40)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Glucose, high (mg/dL)						0 (0)
Level 1 (>200)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Level 2 (>250)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Level 3 (>500)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Calcium, low (mg/dL)						0 (0)
Level 1 (<8.4)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Level 2 (<8)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Level 3 (<7.5)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Calcium, high (mg/dL)						0 (0)
Level 1 (>10.5)	9 (29.0)	2 (15.4)	1 (6.2)	7 (24.1)	0 (0)	9.13 (-13.79, 32.06)
Level 2 (>11)	2 (6.5)	0 (0)	1 (6.2)	0 (0)	0 (0)	-6.25 (-18.11, 5.61)
Level 3 (>12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Magnesium, low (mg/dL)						0 (0)
No data collected	NA	NA	NA	NA	0 (0)	NA
Magnesium, high (mg/dL)						0 (0)
No data collected	NA	NA	NA	NA	0 (0)	NA
Phosphate, low (mg/dL)						0 (0)
Level 1 (<2.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Level 2 (<2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Level 3 (<1.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)

Laboratory Parameter	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Protein, total, low (g/dL)						0 (0)
Level 1 (<6)	2 (6.5)	0 (0)	1 (6.2)	5 (17.2)	0 (0)	-6.25 (-18.11, 5.61)
Level 2 (<5.4)	1 (3.2)	0 (0)	0 (0)	2 (6.9)	0 (0)	0 (0, 0)
Level 3 (<5)	0 (0)	0 (0)	0 (0)	2 (6.9)	0 (0)	0 (0, 0)
Albumin, low (g/dL)						0 (0)
Level 1 (<3.1)	1 (3.2)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Level 2 (<2.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
Level 3 (<2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0, 0)
CPK, high (U/L)						
No data collected	NA	NA	NA	NA	NA	NA
Amylase, high (U/L)						
No data collected	NA	NA	NA	NA	NA	NA
Lipase, high (U/L)						
No data collected	NA	NA	NA	NA	NA	NA

Source: ad b.xpt; software, R; clinical data scientist Table 16, February 16, 2021.
Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Duration is approximately 52 weeks. Participants who completed 48 weeks of treatment and were eligible to receive further treatment could continue in an initial 52-week optional long-term follow-up treatment period, after which eligible participants could continue study treatment beyond Week 52.
Risk difference column shows differences (95% confidence interval) between RWD-MRX and RWD-PLB.
Abbreviations: ARW, after randomized withdrawal; CI, confidence interval; CPK, creatine phosphokinase; LTE- long-term extension; MRX, maralixibat; N, number of patients in treatment arm; n, number of patients meeting criteria; NA, not applicable; OL, open-label; PLB, placebo; RWD, randomized withdrawal; ULN, upper limit of normal

Hematology

No level 3 hematologic abnormalities were observed in the pivotal study (Table 30).

Table 30. Patients with One or More Hematology Analyte Values Outside Specified Levels, Safety Population, Study LUM001-304

Laboratory Parameter	OL-MRX N=31 n/N (%)	RWD-MRX N=13 n/N (%)	RWD-PLB N=16 n/N (%)	ARW-MRX N=29 n/N (%)	LTE-MRX N=23 n/N (%)	Risk Difference (95% CI)
Complete blood count						
WBC, low (cells/µL)						
Level 1 (<3500)	1/31 (3.2)	0/12 (0)	2/16 (12.5)	1/29 (3.4)	0/0 (NA)	-12.50 (-28.70, 3.70)
Level 2 (<3000)	0/31 (0)	0/12 (0)	1/16 (6.2)	0/29 (0)	0/0 (NA)	-6.25 (-18.11, 5.61)
Level 3 (<1000)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
WBC, high (cells/µL)						
Level 1 (>10,800)	6/31 (19.4)	2/12 (16.7)	1/16 (6.2)	7/29 (24.1)	0/0 (NA)	10.42 (-13.78, 34.61)
Level 2 (>13,000)	4/31 (12.9)	0/12 (0)	0/16 (0)	1/29 (3.4)	0/0 (NA)	0 (0, 0)
Level 3 (>15,000)	1/31 (3.2)	0/12 (0)	0/16 (0)	1/29 (3.4)	0/0 (NA)	0 (0, 0)
Hemoglobin, low (g/dL)						
Level 1 (NA)	0/31 (0)	0/12 (0)	0/16 (0)	1/29 (3.4)	0/0 (NA)	0 (0, 0)
Level 2 (>1.5 dec. from baseline)	0/31 (0)	0/12 (0)	0/16 (0)	1/29 (3.4)	0/0 (NA)	0 (0, 0)
Level 3 (>2 dec. from baseline)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Hemoglobin, high (g/dL)						
Level 1 (NA)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Level 2 (>2 inc. from baseline)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Level 3 (>3 inc. from baseline)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Platelets, low (cells/µL)						
Level 1 (<140,000)	1/31 (3.2)	0/12 (0)	1/16 (6.2)	4/29 (13.8)	0/0 (NA)	-6.25 (-18.11, 5.61)
Level 2 (<125,000)	1/31 (3.2)	0/12 (0)	1/16 (6.2)	4/29 (13.8)	0/0 (NA)	-6.25 (-18.11, 5.61)
Level 3 (<1e+05)	1/31 (3.2)	0/12 (0)	1/16 (6.2)	1/29 (3.4)	0/0 (NA)	-6.25 (-18.11, 5.61)
WBC Differential						
Lymphocytes, low (cells/µL)						
Level 1 (<1000)	1/31 (3.2)	0/12 (0)	1/16 (6.2)	2/29 (6.9)	0/0 (NA)	-6.25 (-18.11, 5.61)
Level 2 (<750)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Level 3 (<500)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Lymphocytes, high (cells/µL)						
Level 1 (>4000)	18/31 (58.1)	4/12 (33.3)	4/16 (25.0)	8/29 (27.6)	0/0 (NA)	8.33 (-25.75, 42.41)
Level 2 (>10,000)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Level 3 (>20,000)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)

Laboratory Parameter	OL-MRX N=31 n/N (%)	RWD-MRX N=13 n/N (%)	RWD-PLB N=16 n/N (%)	ARW-MRX N=29 n/N (%)	LTE-MRX N=23 n/N (%)	Risk Difference (95% CI)
Neutrophils, low (cells/µL)						
Level 1 (<2000)	11/31 (35.5)	0/12 (0)	5/16 (31.2)	13/29 (44.8)	0/0 (NA)	-31.25 (-53.96, -8.54)
Level 2 (<1000)	4/31 (12.9)	0/12 (0)	1/16 (6.2)	0/29 (0)	0/0 (NA)	-6.25 (-18.11, 5.61)
Level 3 (<500)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Eosinophils, high (cells/µL)						
Level 1 (>650)	4/31 (12.9)	0/12 (0)	0/16 (0)	7/29 (24.1)	0/0 (NA)	0 (0, 0)
Level 2 (>1500)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Level 3 (>5000)	0/31 (0)	0/12 (0)	0/16 (0)	0/29 (0)	0/0 (NA)	0 (0, 0)
Coagulation studies						
PT, high (seconds)						
Level 1 (>1.1× ULN)	2/31 (6.5)	2/13 (15.4)	0/16 (0)	4/29 (13.8)	0/0 (NA)	15.38 (-4.23, 35.00)
Level 2 (>1.3× ULN)	1/31 (3.2)	0/13 (0)	0/16 (0)	2/29 (6.9)	0/0 (NA)	0 (0, 0)
Level 3 (>1.5× ULN)	0/31 (0)	0/13 (0)	0/16 (0)	1/29 (3.4)	0/0 (NA)	0 (0, 0)
PTT, high (seconds)						
Level 1 (>1× ULN)	10/31 (32.3)	3/13 (23.1)	4/16 (25.0)	20/29 (69.0)	0/0 (NA)	-1.92 (-33.14, 29.30)
Level 2 (>1.21× ULN)	3/31 (9.7)	1/13 (7.7)	1/16 (6.2)	7/29 (24.1)	0/0 (NA)	1.44 (-17.28, 20.16)
Level 3 (>1.41× ULN)	0/31 (0)	0/13 (0)	0/16 (0)	4/29 (13.8)	0/0 (NA)	0 (0, 0)

Source: ad b.xpt; software, R; Clinical Data Scientist Table 20, February 16, 2021.
Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Duration is approximately 52 weeks. Participants who completed 48 weeks of treatment and were eligible to receive further treatment could continue in an initial 52-week optional long-term follow-up treatment period, after which eligible participants could continue study treatment beyond Week 52.
Risk difference column shows differences (95% confidence interval) between RWD-MRX and RWD-PLB.
Abbreviations: ARW, after randomized withdrawal; CI, confidence interval; dec, decrease; inc, increase; LTE, long-term extension; MRX, maralixibat; N, number of patients in treatment arm; n, number of patients meeting criteria; OL, open-label; PLB, placebo; PT, prothrombin time; PTT, partial thromboplastin time; RWD, randomized withdrawal; WBC, white blood cells; ULN, upper limit of normal

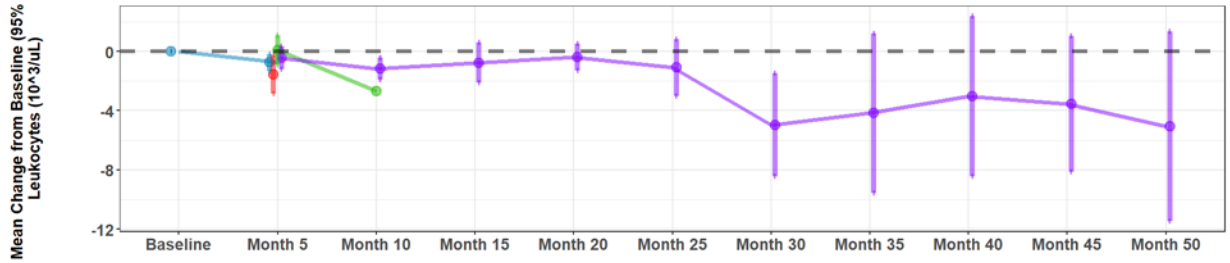
7.6.2. Hematology

Hematological parameters of leucocytes (including lymphocytes and eosinophils), hemoglobin, and platelet counts decreased with higher exposure to maralixibat, as shown in Figure 11.

The following data regarding anemia and thrombocytopenia were reported by the Applicant in response to an IR:

- LUM001-304: Anemia, as defined by hemoglobin <10, was present in 4/31 subjects enrolled in LUM001-304 with mean (range) hemoglobin at time of first diagnosis of 8.9 (7.9, 9.8). None of these subjects had baseline anemia.
- ISS: Thirteen of eighty-six subjects who were treated with maralixibat were diagnosed with anemia, three of whom had anemia at baseline.
- Anemia in subjects with ALGS could have been due to maralixibat, portal hypertension-induced GI blood loss, poor nutrition, or malabsorption-related anemia.
- Using a diagnostic criteria of platelet count <150,000 to diagnose thrombocytopenia, the Applicant reported that 4 of 86 (4.7%) subjects treated with maralixibat had thrombocytopenia at baseline and 22 of 86 (25.6%) developed thrombocytopenia while exposed to maralixibat.
- Thrombocytopenia in subjects with ALGS could have been a consequence of portal hypertension, intercurrent infections, or AEs of other concurrent medicines.

Figure 11. Mean Laboratory (Hematology) Data Change From Baseline Over Time, Safety Population, Study LUM001-304

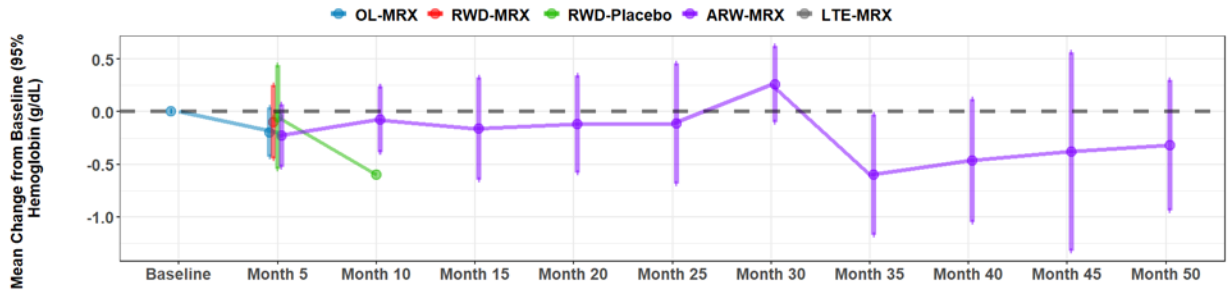


Mean Change from Baseline / Mean Value

	Baseline	Month 5	Month 10	Month 15	Month 20	Month 25	Month 30	Month 35	Month 40	Month 45	Month 50
OL-MRX	0/7.98	-0.75/7.12	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
RWD-MRX	NA/NA	-1.56/7.64	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
RWD-Placebo	NA/NA	0.09/6.99	-2.7/2.7	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
ARW-MRX	NA/NA	-0.43/7.51	-1.16/6.72	-0.76/6.73	-0.38/6.49	-1.1/7.79	-4.96/6.76	-4.16/6.36	-3.02/7.65	-3.54/6.22	-5.06/6.66
LTE-MRX	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA

Number of Patients with Data

	Baseline	Month 5	Month 10	Month 15	Month 20	Month 25	Month 30	Month 35	Month 40	Month 45	Month 50
OL-MRX	31	31	0	0	0	0	0	0	0	0	0
RWD-MRX	0	12	0	0	0	0	0	0	0	0	0
RWD-Placebo	0	15	1	0	0	0	0	0	0	0	0
ARW-MRX	0	28	29	16	15	14	3	3	3	3	3
LTE-MRX	0	0	0	0	0	0	0	0	0	0	0

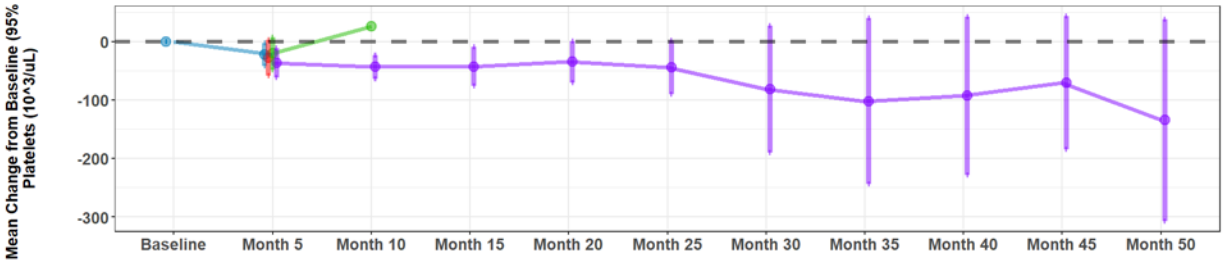


Mean Change from Baseline / Mean Value

	Baseline	Month 5	Month 10	Month 15	Month 20	Month 25	Month 30	Month 35	Month 40	Month 45	Month 50
OL-MRX	0/13	-0.2/12.79	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
RWD-MRX	NA/NA	-0.1/12.65	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
RWD-Placebo	NA/NA	-0.05/13.03	-0.6/13	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
ARW-MRX	NA/NA	-0.23/12.61	-0.07/12.77	-0.16/12.87	-0.12/13.06	-0.11/12.86	0.26/13.1	-0.6/12.46	-0.47/12.5	-0.38/12.62	-0.32/12.52
LTE-MRX	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA

Number of Patients with Data

	Baseline	Month 5	Month 10	Month 15	Month 20	Month 25	Month 30	Month 35	Month 40	Month 45	Month 50
OL-MRX	31	31	0	0	0	0	0	0	0	0	0
RWD-MRX	0	12	0	0	0	0	0	0	0	0	0
RWD-Placebo	0	15	1	0	0	0	0	0	0	0	0
ARW-MRX	0	28	29	16	15	14	3	3	3	3	3
LTE-MRX	0	0	0	0	0	0	0	0	0	0	0



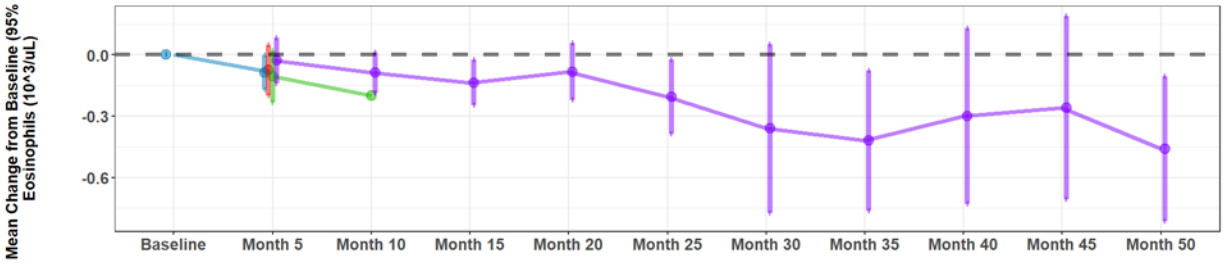
Mean Change from Baseline / Mean Value

	Baseline	Month 5	Month 10	Month 15	Month 20	Month 25	Month 30	Month 35	Month 40	Month 45	Month 50
OL-MRX	0/317.71	-22.56/296.01	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
RWD-MRX	NA/NA	-27.67/316.33	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
RWD-Placebo	NA/NA	-20.4/283.07	26/189	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
ARW-MRX	NA/NA	-36.7/287.67	-43.82/275.48	-42.84/279.19	-34.67/272.73	-44.57/306.76	-81.6/314.6	-102.2/227.2	-93/264.33	-70.4/276	-134.6/261.6
LTE-MRX	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA

Number of Patients with Data

	Baseline	Month 5	Month 10	Month 15	Month 20	Month 25	Month 30	Month 35	Month 40	Month 45	Month 50
OL-MRX	31	31	0	0	0	0	0	0	0	0	0
RWD-MRX	0	12	0	0	0	0	0	0	0	0	0
RWD-Placebo	0	15	1	0	0	0	0	0	0	0	0
ARW-MRX	0	27	29	16	15	14	3	3	3	3	3
LTE-MRX	0	0	0	0	0	0	0	0	0	0	0

OL-MRX RWD-MRX RWD-Placebo ARW-MRX LTE-MRX



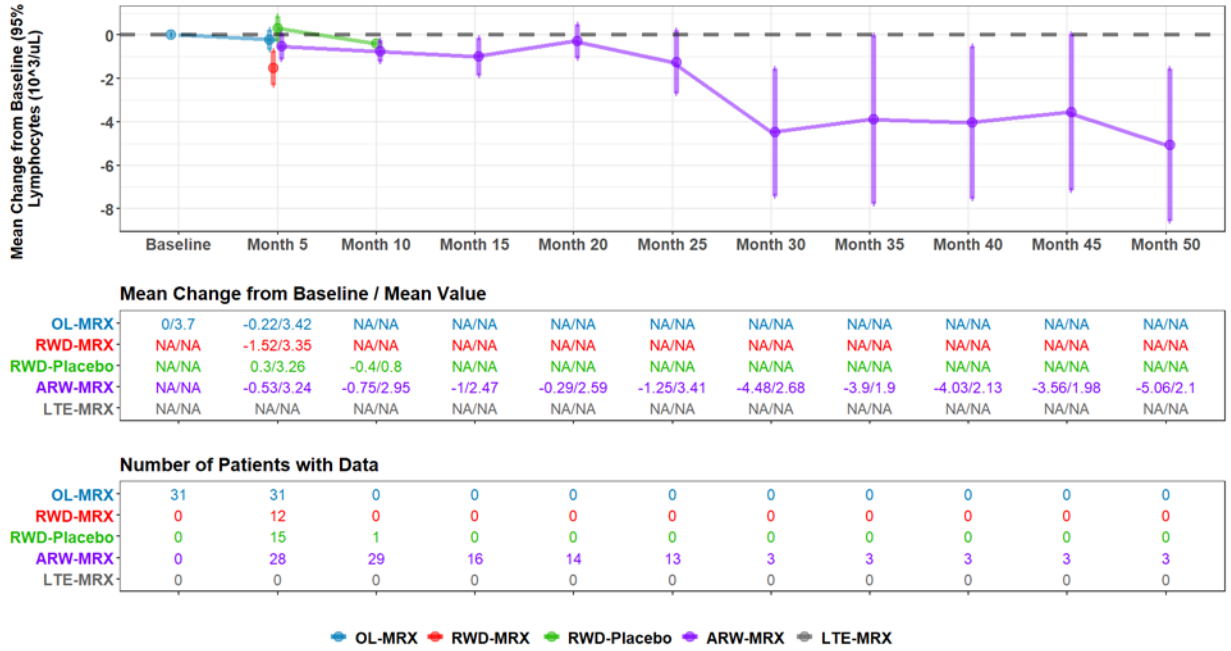
Mean Change from Baseline / Mean Value

	Baseline	Month 5	Month 10	Month 15	Month 20	Month 25	Month 30	Month 35	Month 40	Month 45	Month 50
OL-MRX	0/0.34	-0.09/0.26	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
RWD-MRX	NA/NA	-0.08/0.28	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
RWD-Placebo	NA/NA	-0.11/0.26	-0.2/0	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
ARW-MRX	NA/NA	-0.03/0.33	-0.09/0.26	-0.14/0.26	-0.08/0.3	-0.2/0.31	-0.36/0.28	-0.42/0.16	-0.3/0.27	-0.26/0.22	-0.46/0.18
LTE-MRX	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA

Number of Patients with Data

	Baseline	Month 5	Month 10	Month 15	Month 20	Month 25	Month 30	Month 35	Month 40	Month 45	Month 50
OL-MRX	31	31	0	0	0	0	0	0	0	0	0
RWD-MRX	0	12	0	0	0	0	0	0	0	0	0
RWD-Placebo	0	15	1	0	0	0	0	0	0	0	0
ARW-MRX	0	28	29	16	14	13	3	3	3	3	3
LTE-MRX	0	0	0	0	0	0	0	0	0	0	0

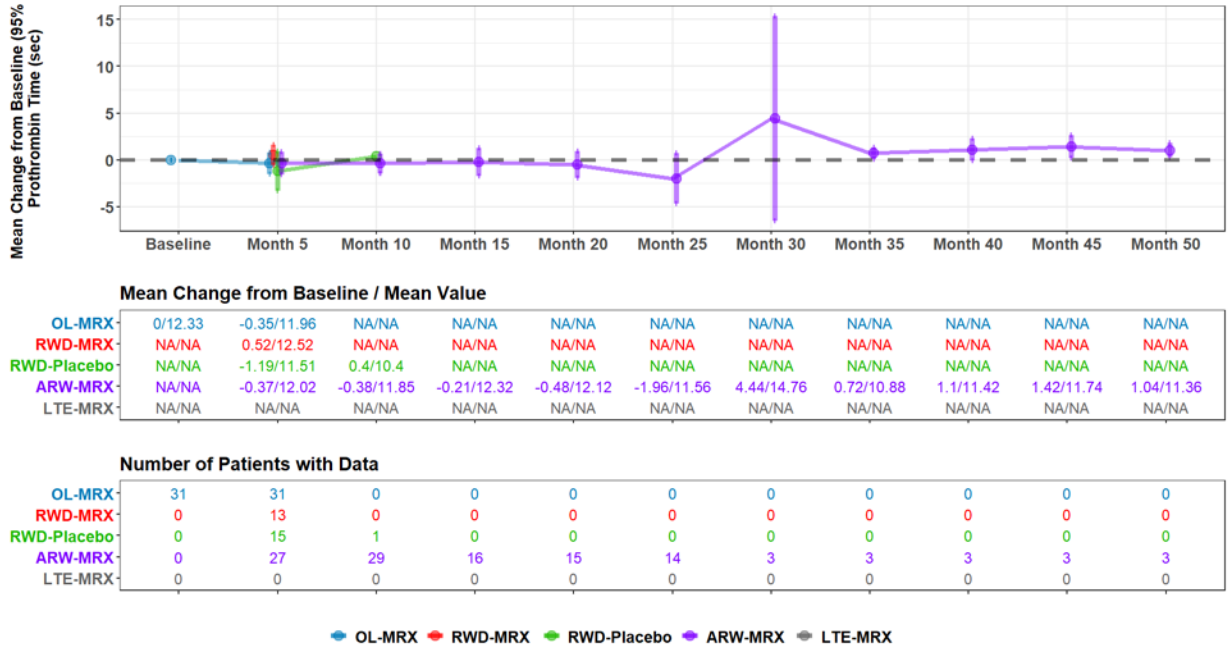
OL-MRX RWD-MRX RWD-Placebo ARW-MRX LTE-MRX



Source: Clinical data scientist's Figure 7; February 16, 2021.
Abbreviations: ARW, after randomized withdrawal; LTE, long-term extension; MRX, maralixibat; NA, not applicable; OL, open-label; RWD, randomized withdrawal (Source: Figure 7; CDS February 16, 2021)

One subject in LUM001-304 had an increased INR while on maralixibat at 400 mcg/kg/day, without associated bleeding. However, 13 subjects in the ISS had an increased INR, 4 of whom had associated bleeding (2 GI bleeding and 2 epistaxis). See Figure 12.

Figure 12. Prothrombin Change From Baseline Over Time, Safety Population, Study LUM001-304



Source: Clinical data scientist's Figure 7; February 16, 2021.
Abbreviations: ARW, after randomized withdrawal; LTE, long-term extension; MRX, maralixibat; NA, not applicable; OL, open-label; RWD, randomized withdrawal

7.6.3. Liver Biochemistry

Liver parameters assessment in RWD-maralixibat and RWD-PLB were compared and the level 3 findings of subjects with laboratory results outside the specified levels revealed:

- For RWD-maralixibat and RWD-PLB, 3/13 (23.1%) and 1/16 (6.2%) subjects had ALT abnormalities. No patient in RWD-maralixibat and RWD-PLB met the criteria for level 3 AST abnormalities. For RWD-maralixibat and RWD-PLB, 1/13 (7.7%) and 0/16 subjects had alkaline phosphatase abnormalities. For RWD-maralixibat and maralixibat-PLB, 7/13 (53.8%) and 8/16 (50%) subjects had bilirubin abnormalities. See Table 31.

In contrast the following subjects experienced level 2 liver test abnormalities:

- For RWD-maralixibat and maralixibat -PLB, 11/13 (84.6%) and 9/16 (56.2%) subjects had ALT abnormalities. For RWD-maralixibat and RWD-PLB 5/13 (38.5%) and 3/16 (18.8%) subjects had AST abnormalities. For RWD-maralixibat and RWD-PLB, 2/13 (15.4 %) and 1/16 (6.2%) subjects had alkaline phosphatase abnormalities. For RWD-maralixibat and RWD-PLB, 9/13 (69.2%) and 8/16 (50%) subjects had bilirubin abnormalities. See Table 31.

Table 31. Patients With One or More Liver Biochemistry Analyte Values Outside Specified Levels, Safety Population, Study LUM001-304

Laboratory Parameter	OL-MRX N=31 n (%)	RWD-MRX N=13 n (%)	RWD-PLB N=16 n (%)	ARW-MRX N=29 n (%)	LTE-MRX N=23 n (%)	Risk Difference (95% CI)
Alkaline phosphatase, high (U/L)						
Level 1 (>1.5× ULN)	11 (35.5)	3 (23.1)	4 (25.0)	14 (48.3)	0 (0)	-1.92 (-33.14, 29.30)
Level 2 (>2× ULN)	5 (16.1)	2 (15.4)	1 (6.2)	7 (24.1)	0 (0)	9.13 (-13.79, 32.06)
Level 3 (>3× ULN)	1 (3.2)	1 (7.7)	0 (0)	1 (3.4)	0 (0)	7.69 (-6.79, 22.18)
Alanine aminotransferase, high (U/L)						
Level 1 (>3× ULN)	30 (96.8)	12 (92.3)	15 (93.8)	29 (100)	0 (0)	-1.44 (-20.16, 17.28)
Level 2 (>5× ULN)	22 (71.0)	11 (84.6)	9 (56.2)	25 (86.2)	0 (0)	28.37 (-2.87, 59.60)
Level 3 (>10× ULN)	6 (19.4)	3 (23.1)	1 (6.2)	13 (44.8)	0 (0)	16.83 (-8.97, 42.62)
Aspartate aminotransferase, high (U/L)						
Level 1 (>3× ULN)	18 (58.1)	7 (53.8)	6 (37.5)	21 (72.4)	0 (0)	16.35 (-19.67, 52.36)
Level 2 (>5× ULN)	7 (22.6)	5 (38.5)	3 (18.8)	9 (31.0)	0 (0)	19.71 (-12.93, 52.35)
Level 3 (>10× ULN)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0, 0)
Bilirubin, total, high (mg/dL)						
Level 1 (>1.5× ULN)	20 (64.5)	9 (69.2)	9 (56.2)	20 (69.0)	0 (0)	12.98 (-21.95, 47.91)
Level 2 (>2× ULN)	19 (61.3)	9 (69.2)	8 (50.0)	19 (65.5)	0 (0)	19.23 (-15.84, 54.30)
Level 3 (>3× ULN)	17 (54.8)	7 (53.8)	8 (50.0)	16 (55.2)	0 (0)	3.85 (-32.69, 40.38)

Source: Clinical data scientist's Table 20; February 16, 2021; ad b.xpt; software, R.
Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Duration is approximately 52 weeks. Participants who completed 48 weeks of treatment and were eligible to receive further treatment could continue in an initial 52-week optional long-term follow-up treatment period, after which eligible participants could continue study treatment beyond Week 52.
Risk difference column shows differences (95% confidence interval) between RWD-MRX and RWD-PLB.
Abbreviations: ARW, after randomized withdrawal; CI, confidence interval; LTE, long-term extension; MRX, maralixibat; OL, open-label; RWD, randomized withdrawal; ULN, upper limit of normal

7.6.4. Safety Findings and Concerns, ISS

7.6.4.1. Overall TEAE Summary, ISS Population

The majority of the subjects in the ISS population experienced at least one AE (83/84, 98.8%). The most common AEs were diarrhea (41/84, 48.8%), abdominal pain (40/84, 47.6%), and liver injury as evidenced by ALT (15/84, 17.9%) or AST (10/84, 11.9%) elevations. All subjects who received maralixibat at 280 mcg/kg and ≤140 mcg/kg experienced an AE, compared to 37/84 (97.4%) of those who received 280 mcg/kg. Diarrhea and abdominal pain were most frequent with maralixibat at >280 mcg/kg (22/38 [61.1%] for diarrhea and 23/38 [63.9%] for abdominal pain) (Table 32).

Table 32. Treatment-Emergent Adverse Events, Integrated Summary of Safety Population

Preferred Term	MRX ≤140 mcg N=10 n (%)	MRX 280 mcg N=38 n (%)	MRX >280 mcg N=36 n (%)	Overall MRX N=84 n (%)
Any AE	10 (100)	37 (97.4)	36 (100)	83 (98.8)
Diarrhea	2 (20)	17 (44.7)	22 (61.1)	41 (48.8)
Abdominal pain	3 (30)	14 (36.8)	23 (63.9)	40 (47.6)
ALT and AST increased	4 (40)	15 (39.5)	6 (16.7)	25 (29.8)
Vitamin E decreased	0	0	1(2.8)	1 (1.2)
Vitamin K deficiency	0	1 (2.6)	0	1 (1.2)
Vomiting	2 (20)	13 (34.2)	17 (47.2)	32 (38.1)
INR increased	1 (10)	7 (38)	3 (8.3)	11 (13.1)
Thrombocytopenia	0	1 (2.6)	0	1 (1.2)
Fracture	0	4 (10.5)	3 (8.3)	7 (8.3)
Growth retardation	0	1 (2.6)	1 (2.8)	2 (2.4)

Source: Clinical data scientist's Table 13; March 17, 2021.
Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; MRX, maralixibat

TEAEs Resulting in Discontinuation

Six subjects discontinued maralixibat during Study LUM001-304 because hyperbilirubinemia (one), increased transaminases (two), acute kidney injury (one), extradural and subdural hematomas (one), and staphylococcal infection (one). Three subjects had concurrent diarrhea that contributed to discontinuation of therapy.

TEAEs Resulting in Dose Modification

Two subjects in LUM001-304 underwent dose reduction due to increased transaminases and diarrhea, respectively.

- Subject 304-(b) (6): Increased transaminases (ALT 471 on Day 1360 from baseline of 156 U/L) at 800 mcg/kg/day maralixibat, which was consequently reduced to 400 mcg/kg/day from Days 1361 to 1799 and subsequently to 140 mcg/kg/day from Days 1800 to 1980.
- Subject 304-(b) (6) experienced diarrhea on Day 925 on maralixibat at 540 mcg/kg/day. The maralixibat dose was reduced on Day 938 to 400 mcg/kg/day, which was maintained until Day 1390.

Dose Interruptions

Dose interruptions occurred in four subjects in LUM001-304 for procedures (two), hypertension (one), multiple injuries accompanied by splenic rupture (one), increased transaminases (one), bone fracture (one), and abdominal pain (one). See Table 33.

Table 33. Adverse Events, ISS Population

Event Category	≤ 140 µg N=10 n (%)	280 µg N=38 n (%)	>280 µg N=36 n (%)	Overall MRX N=84 n (%)
SAE	3 (30.0)	6 (15.8)	16 (44.4)	25 (29.8)
SAEs with fatal outcome	0	0	0	0
Life-threatening SAEs	0	0	4 (11.1)	4 (4.8)
AE leading to permanent discontinuation of study drug	2 (20.0)	5 (13.2)	6 (16.7)	13 (15.5)
AE leading to dose modification of study drug	2 (20.0)	5 (13.2)	10 (27.8)	17 (20.2)
AE leading to interruption of study drug	2 (20.0)	3 (7.9)	8 (22.2)	13 (15.5)
AE leading to reduction of study drug	0	2 (5.3)	3 (8.3)	5 (6.0)
AE leading to dose delay of study drug	0	0	0	0
AE	10 (100)	37 (97.4)	36 (100)	83 (98.8)
Death	0	0	0	0
Life-threatening	0	0	5 (13.9)	5 (6.0)
Severe	5 (50.0)	12 (31.6)	11 (30.6)	28 (33.3)
Moderate	5 (50.0)	18 (47.4)	15 (41.7)	38 (45.2)
Mild	0	7 (18.4)	5 (13.9)	12 (14.3)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as AEs with a start date on or after the first dose of study drug and started prior to the last dose of study drug plus 14 days. For subjects with >14 days of study drug interruption/withdrawal, the definition of a TEAE considers both the date of the last dose.
Duration is 18 weeks for the LUM001-304, approximately 254 weeks for the LUM001-303, approximately 198 weeks for the LUM001-305.
Abbreviations: AE, adverse event; N, number of patients in treatment arm; n, number of patients with at least one event; SAE, serious adverse event; µg, microgram.

Source: Clinical data scientist's Table 6; March 17, 2021.

7.6.4.2. Deaths, ISS Population

No deaths were reported in the ISS population.

Adverse Events Leading to Discontinuation

No AE occurred in RWD-maralixibat or RWD-PLB that led to discontinuation. Overall, two subjects in LTE-maralixibat and one in OL-maralixibat experienced an AE that led to discontinuation of maralixibat.

Portal Hypertension

In a response to an IR, the Applicant reported that four subjects were diagnosed with cirrhosis or portal hypertension, one of which was deemed possibly related to maralixibat in one, and another who had a concurrent diagnosis of a liver lesion.

7.6.4.3. Dropouts and/or Discontinuations Due to AEs, ISS

Twenty-six subjects experienced AEs that led to dose modifications, 12 of which were related to liver abnormalities (1 attributed to a diagnosis of autoimmune hepatitis) and 6 related to GI

abnormalities (1 vomiting, 3 abdominal pain, 2 diarrhea); 1 increase in INR; 1 GI bleeding associated with anemia; 1 from gastrostomy; one from phimosis; 1 related to hypertension (and acute kidney injury); 1 hemorrhagic shock (splenic rupture from trauma); 1 due to forearm fracture; 1 due to subdural/epidural hemorrhage; and 1 related to staphylococcal infection.

One Hundred Twenty-Day Safety Update

No new safety concerns were identified after our thorough review of the 120-day safety update submitted with the NDA.

7.7. Key Review Issues Relevant to Evaluation of Risk

7.7.1. Liver Test Abnormalities

Issue

Liver test abnormalities, consisting of ALT, AST, bilirubin, and ALP elevations, were observed across treatment groups in LUM001-304 and the ISS. Most abnormalities were related to increased ALT and AST.

Background

ALGS is characterized by cholestatic liver injury in the background of multisystemic syndromic abnormalities. In addition to an elevation in total bilirubin, there is typically a baseline elevation in ALT and AST. The median ALT and AST are usually >100, potentially with significant within-patient biologic variations (e.g., ALT varied from 56% lower to 129% higher in one series of patients with ALGS) (Kamath et al. 2020). Laboratory findings of patients with ALGS typically reveal higher levels of bilirubin and pediatric end-stage liver disease scores than age-matched patients with other congenital liver diseases, such as biliary atresia (Kamath et al. 2012).

Baseline values from LUM001-304 (source, Table 14.1.3.1 Study Report Body Chapter, LUM001-304 Tables and Figures) demonstrate elevations in ALT, AST, total bilirubin, and ALP. One subject discontinued MRX due to hyperbilirubinemia approximately 6 months after treatment initiation, and two other subjects discontinued treatment due to ALT elevations in the LTE. Data from the ISS revealed that 12 subjects had liver-related disorders that led to discontinuation of maralixibat, 9 of which were due to abnormal AST or ALT, 1 to a diagnosis of autoimmune hepatitis, and 2 to hyperbilirubinemia. ALT elevations were generally of greater magnitude than elevations in AST and were typically the indication for treatment interruption or discontinuation. Five subjects underwent liver transplantation (during the study) for progression of ALGS (three), hepatocellular carcinoma (one), and pruritus (one) during the mean of 1230 days (range, 437 to 1718 days), none of which were due to acute liver failure.

Due to the underlying baseline liver test elevations and fluctuations therein, it is difficult to attribute treatment-emergent liver test abnormalities to study drug in uncontrolled studies. However, there is evidence that maralixibat is associated with a potential signal of DILI, as supported by ALT elevations in placebo-controlled studies of subjects with baseline normal liver enzymes. ALT elevations were found in healthy volunteer subjects after 4 weeks of exposure to

maralixibat (9%) compared with placebo (5%) (Study NB4-02-06-004) and after 7 days of exposure in obese subjects (mean [SD] increase in ALT 29.5 (36.0) U/L in the maralixibat arm, compared with ALT 1.1 [6.2] U/L in the placebo arm [Study SHP625-101]; Summary of Clinical Safety). Although elevations in ALT were modest in these two studies, exposure was short (7 to 28 days) and baseline liver function was normal.

In addition, an independent adjudication committee reviewed 27 cases of significant ALT elevation, TB elevation, or discontinuation due to liver AEs in the ALGS development program. Of the 27 cases, 1 was deemed probable, 6 possible, and 20 as unlikely related to maralixibat (Liver Safety Data Adjudication Report, Final Version 1.0_16 June 2020).

Table 34. Abnormal Baseline Laboratory Values in Study LUM001-304

	Open-Label Phase	Randomized Withdrawal (RW) Phase		After RW Phase	
	(Day 1-Week 18)	(Week 19-Week 22)		(Week 23-Week 48)	(Week >48)
Parameter Category	MRX (N=31)	MRX (N=13)	Placebo (N=16)	MRX (N=29)	MRX (N=23)
Alanine aminotransferase (U/L)					
n	31	13	16	29	23
Mean	181.0	217.8	147.0	178.7	182.6
SD (SE)	108.56 (19.50)	149.93 (41.58)	54.60 (13.65)	111.86 (20.77)	123.52 (25.76)
Median	171.0	196.0	143.5	164.0	164.0
Minimum, max	41, 626	41, 626	54, 249	41, 626	41, 626
Aspartate aminotransferase (U/L)					
n	31	13	16	29	23
Mean	167.7	172.4	146.8	158.2	155.7
SD (SE)	75.87 (13.63)	76.12 (21.11)	61.34 (15.34)	68.32 (12.69)	72.38 (15.09)
Median	161.0	183.0	135.0	158.0	158.0
Min, max	32, 350	52, 350	32, 296	32, 350	32, 350

Source: Table 14.1.3.1, LUM001-304 Clinical Study Report, Tables & Figures

Table 35. Disease History and Baseline Disease Characteristics Safety Population

	Open-Label Phase	Randomized Withdrawal (RW) Phase		After RW Phase	
	(Day 1-Week 18)	(Week 19-22)		(Week 23-48)	(Week >48)
Parameter Category	MRX (N=31)	MRX (N=13)	Placebo (N=16)	MRX (N=29)	MRX (N=23)
Gamma glutamyl transferase (U/L)					
n	31	13	16	29	23
Mean	508.4	613.9	404.0	498.1	408.6
SD (SE)	389.35 (69.93)	482.48 (133.82)	300.19 (75.05)	399.16 (74.12)	278.64 (58.10)
Median	419.0	463.0	310.5	386.0	354.0
Min, max	86, 1545	86, 1545	99, 1021	86, 1545	86, 1021
Total bilirubin (mg/dL)					
n	31	13	16	29	23
Mean	6.09	6.52	4.83	5.59	4.09
SD (SE)	5.781 (1.038)	6.571 (1.822)	4.265 (1.066)	5.383 (1.000)	3.947 (0.823)
Median	4.60	4.60	2.85	3.80	1.90
Min, max	0.4, 20.5	0.5, 20.5	0.4, 13.4	0.4, 20.5	0.4, 13.4

Source: Source: Table 14.1.3.1, LUM001-304 Clinical Study Report, Tables & Figures

Assessment

Review of the data across treatment groups from LUM001-304 and ISS revealed abnormal liver tests (AST, ALT, ALP, and bilirubin). Regarding LUM001-304, two of six SAEs that were due to elevated AST and ALT and one increased bilirubin led to discontinuation of maralixibat.

In an IR response, the Applicant stated that in Study LUM001-304, the mean (range) time to substantial increased liver tests (1.5× baseline) was 158.6 days (range, 20 to 330 days). Five subjects met six prespecified event criteria for dose adjustment (three) or discontinuation (three, including one who had prior dose reduction). Four subjects (two treated with maralixibat at 400 and 800 mcg/kg/day) displayed increased ALT (mean [range] 627 [471 to 815] U/L). One subject had hyperbilirubinemia to 41.9 mg/d on maralixibat at 400 mcg/kg/day on Day 270. All dose modifications for LUM001-304 that met prespecified liver test thresholds occurred at maralixibat doses of 400 mcg/kg/day (three) and 800 mcg/kg/day (two).

Study discontinuations occurred in three subjects, due to bilirubin 41.9 mg/dL and ALT 598 and 815 U/L.

Subject 304-(b) (6) was treated with maralixibat at 400 mcg/kg/day on Day 229, and drug was discontinued on Day 270.

Subject 304-(b) (6) had ALT increased to 297 U/L on Day 1773 while receiving maralixibat at 400 mcg/kg/day; therefore, dose reduction was commenced, resulting in an intermittent and non-sustained ALT response upon increasing the maralixibat dose.

Subject 304-(b) (6): ALT increase was observed as the dose of maralixibat increased, without an associated response of ALT to dose reduction. However, while the subject was treated with placebo, there was a rebound increase in ALT as the maralixibat dose increased to 400 mcg/kg/day.

Subject 304-(b) (6): Variable increase of ALT from baseline of unclear dose association.

In the ISS, 17 SAEs in 11 subjects were associated with dose adjustments or discontinuations due to abnormal transaminases and in 2 subjects to hyperbilirubinemia. Liver test abnormalities represented the third most common AESI across studies in the ISS.

The prespecified thresholds for discontinuation of maralixibat or placebo in LUM001-301, -303, -304, and -305 are listed in Table 36.

Table 36. Discontinuation Thresholds, Studies LUM001-301, -303, -304, and -305

Baseline Test	Change Observed
ALT	ALT ≥20× ULN
Bilirubin 1-10 mg/dL	5 mg increase and 2× increase over baseline level
Total bilirubin >10 mg/dL	2× increase over baseline level

Source: Response by Applicant to Information Request SDN 35, dated June 28, 2021.
Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal

Biological Plausibility

BA and bile formation are important for intestinal lipid digestion and absorption, cholesterol homeostasis, lipid soluble substances (such as FSVs) and heavy metals (Hofmann 1967). When BA homeostasis is disturbed, liver disease, malabsorption, and gallstones can ensue. Approximately 95% of BAs are reabsorbed in the small intestine via sodium-dependent apical bile acid transporters (Wong et al. 1994). When this enterohepatic circulation is disturbed, changes in the composition of bile can lead to accumulation of toxic bile salts that may injure the liver.

Conclusion

The pivotal study was of short duration and the data from the ISS are uncontrolled, therefore, there is insufficient controlled data from which to draw a conclusion regarding the propensity for maralixibat to result in liver injury with long-term use. Although the data from the ISS are not controlled, there appears to be a safety signal possibly not directly attributable to the actions of maralixibat. Instead, it could be a result of progression of ALGS and long-term effects on bile.

Additional studies in the postmarketing period will be commitments to provide long-term safety information based on the preapproval signals noted in this population of patients (who are at increased risk of abnormal liver tests) due to background of liver test fluctuations over time.

7.7.2. Diarrhea, Vomiting, Abdominal Pain

Issue

Diarrhea, vomiting, and abdominal pain were the most frequent adverse reactions. IBAT inhibitors, such as maralixibat, increase the excretion of BAs. This results in choleretic diarrhea due to an increased colonic BA concentration. Therefore, diarrhea is one of the most common AESIs of IBAT inhibitors.

Background

Gastrointestinal AEs (diarrhea, vomiting, abdominal pain) were the second most common AEs leading to interruptions or dose adjustments of maralixibat. Diarrhea was the most common AESI in LUM001-304 (21/29 patients) and led to hospitalization in 2 of 29 subjects. It was also the most common AE in the ISS population.

During LUM001-304, three of the six (50%) SAEs that led to discontinuation of maralixibat were due to diarrhea. Most cases were mild to moderate; however, two subjects treated with maralixibat experienced severe diarrhea leading to hospitalization. Cholerheic diarrhea promoted by maralixibat is the probable mechanism of diarrhea in these cases.

If BAs—especially dihydroxy BA, chenodeoxycholic acids, and deoxycholic acids—are not absorbed in the small intestine, they accumulate in the colon and cause loose, watery diarrhea.

The potential etiopathogenesis of BA-induced diarrhea is:

- Defective feedback inhibition of BA biosynthesis by fibroblast growth factor-19. An inverse relationship exists between fibroblast growth factor-19 and serum C4 (a surrogate marker of hepatic BA synthesis) in patients with BA-induced diarrhea (Lundåsen et al. 2006).
- Genetic mutations in apical sodium-dependent BA transporter (Oelkers et al. 1997).

BA malabsorption and associated diarrhea (cholerheic diarrhea) is linked to abdominal boating and discomfort. Cholerheic diarrhea appears to be precipitated and maintained by the following effects of increased colonic BAs:

- Induction of secretion of sodium and water.
- Increased colonic motility.
- Stimulation of defecation.
- Induction of mucous secretion.
- Injury to the mucosa, increasing mucosal permeability.

Assessment

Cholerheic diarrhea promoted by maralixibat is the probable mechanism of diarrhea in these cases. An assessment of fibroblast growth factor-19 and serum C4 could have assisted in verifying the diagnosis.

Twenty-six subjects across the drug development (ISS) program required drug interruptions, reduction, or withdrawal, 3/26 were due to abdominal pain and 2/26 to diarrhea. These findings do not show a strong relationship to maralixibat, since most data are uncontrolled, however, they are consistent with the AESIs associated with IBAT inhibitors. Hence, close monitoring and dose modification will be recommended for patients who experience GI AEs, such as diarrhea, abdominal pain, and vomiting**.**

Conclusion

BA malabsorption due to maralixibat has the potential to result in GI symptoms. The most serious GI-related AESI across treatment groups was diarrhea (likely cholerheic diarrhea), which resulted in net secretion of water. Cholerheic diarrhea can result in volume depletion and electrolyte abnormalities. Abdominal pain, vomiting, and nausea were also associated with drug interruptions, dose adjustments, and interventions (hospitalization for IV fluids and antiemetics) and vomiting could have arisen from the bloating associated with increased secretion of sodium and water, which is worsened by increased colonic motility. Volume depletion due to voluminous diarrhea is a potential risk of maralixibat.

7.7.3. Fat-Soluble Vitamin Deficiency

Issue

FSV deficiency was present at baseline in a majority of subjects. A large portion of subjects required increased supplementation (either increased dosing of the same vitamins or initiation of new vitamin supplementation) during the study.

Background

FSV is an established complication of ALGS, and FSV levels are monitored and supplemented in patients with ALGS and other pediatric cholestatic diseases.

Upon review of the data from LUM001-304, all subjects required FSV supplementation either before or after being treated with maralixibat. However, vitamin D was the most common FSV deficiency: 23 of 31 (74.2%) subjects required vitamin D replacement therapy at baseline. Increased doses of vitamin D were administered to 17 of 31 (67.7%) subjects during LUM001-304. Six subjects at Week 48 were diagnosed with insufficient vitamin D (<20 ng/mL).

Seventy-six subjects across the ISS drug development program were administered vitamin D for the first time or required increased doses of vitamin D. Eight of them were diagnosed with bone fractures. Most maralixibat dose modifications or decreases were associated with doses of ≥280 mcg/kg/day. See Table 37 for maralixibat doses and vitamin D supplementation.

Table 37. Summary of Prior Medications (Excluding Anti-Pruritis Agents), Safety Population

	Open-Label Phase	Randomized Withdrawal (RW) Phase		After RW Phase	
	(Day 1 - Week 18)	(Week 19 - Week 22)		(Week 23 - Week 48)	(Week > 48)
ATC Preferred Term [1]	MRX (N=31) n (%)	MRX (N=13) n (%)	Placebo (N=16) n (%)	MRX (N=29) n (%)	MRX (N=23) n (%)
VITAMINS	26 (83.9%)	12 (92.3%)	12 (75.0%)	24 (82.8%)	18 (78.3%)
ALFACALCIDOL	2 (6.5%)	1 (7.7%)	1 (6.3%)	2 (6.9%)	2 (8.7%)
ASCORBIC ACID;CALCIUM PANTOTHENATE;ERGOCALCIFEROL;NICOTINAMIDE;PYRIDOXINE HYDROCHLORIDE;RETINOL;RIBOFLAVIN;THIAMINE MONONITRATE	1 (3.2%)	1 (7.7%)	0	1 (3.4%)	1 (4.3%)
ASCORBIC ACID;ERGOCALCIFEROL;NICOTINAMIDE;PYRIDOXINE HYDROCHLORIDE;RETINOL PALMITATE;RIBOFLAVIN;THIAMINE HYDROCHLORIDE	2 (6.5%)	1 (7.7%)	1 (6.3%)	2 (6.9%)	2 (8.7%)
ASCORBIC ACID;ERGOCALCIFEROL;RETINOL;TOCOPHEROL	1 (3.2%)	1 (7.7%)	0	1 (3.4%)	0
CALCIFEDIOL	6 (19.4%)	4 (30.8%)	2 (12.5%)	6 (20.7%)	2 (8.7%)
COLECALCIFEROL	4 (12.9%)	2 (15.4%)	1 (6.3%)	3 (10.3%)	3 (13.0%)
ERGOCALCIFEROL	1 (3.2%)	1 (7.7%)	0	1 (3.4%)	1 (4.3%)
MULTIVITAMINS, PLAIN	4 (12.9%)	2 (15.4%)	1 (6.3%)	3 (10.3%)	1 (4.3%)
OMEGA-3 FATTY ACIDS;VITAMIN E NOS	1 (3.2%)	1 (7.7%)	0	1 (3.4%)	1 (4.3%)
RETINOL	6 (19.4%)	2 (15.4%)	4 (25.0%)	6 (20.7%)	4 (17.4%)
RETINOL ACETATE	1 (3.2%)	0	0	0	0
TOCOFERSOLAN	9 (29.0%)	3 (23.1%)	6 (37.5%)	9 (31.0%)	6 (26.1%)
TOCOPHEROL	9 (29.0%)	5 (38.5%)	3 (18.8%)	8 (27.6%)	7 (30.4%)
VITAMIN A AND D, INCL. COMBINATIONS OF THE TWO	1 (3.2%)	0	0	0	0
VITAMIN D NOS	9 (29.0%)	2 (15.4%)	7 (43.8%)	9 (31.0%)	9 (39.1%)

Note: Percentages are 100*n/N. Actual treatment groups are presented. Subjects were counted only once for each Anatomical Therapeutic Chemical (ATC) or Preferred Term. Medications that are stopped prior to the time of informed consent were not recorded and are thus not included in this summary.
[1] Medications were coded using WHO- DD (Enhanced version Sept 2019) ATC Level 2 and Chemical Substance (generic name).
SOURCE: Listing 16.4.3, 16.4.4
Source: Table 14.1.5.2, Summary of Prior Medications, LUM001-304 clinical study report, Tables and Figures.
Abbreviations: ATC, anatomical therapeutic chemical; MRX, maralixibat

Table 38. Subjects Receiving Concomitant Vitamin D Medications and Vitamin D-Containing Formulations, Open-Label/Extension Safety Population

	ISS			
Preferred Term	MRX ≤140 µg/kg (N=10)	MRX 280 µg/kg (N=38)	MRX >280 µg/kg (N=36)	Overall MRX (N=84)
Vitamin D formulations				
Alfacalcidol	0	4 (10.5)	5 (13.9)	9 (10.7)
Ascorbic acid, ergocalciferol, retinol, tocopherol, zinc	0	0	1 (2.8)	1 (1.2)
Calcifediol	0	0	6 (16.7)	6 (7.1)
Calcitriol	1 (10)	4 (10.5)	0	5 (6.0)
Calcium carbonate, ergocalciferol, retinol	1 (10)	1 (2.6)	0	2 (2.4)
Chloride, cyanocobalamin, ergocalciferol, retinol	0	0	1 (2.8)	1 (1.2)
Colecalciferol	5 (50)	7 (18.4)	11 (30.6)	23 (27.4)
Ergocalciferol	3 (30)	9 (23.7)	2 (5.6)	14 (16.7)

Source: Table 1.6.1.3. Concomitant Medication-Open Label/Extension Safety Population
Abbreviations: ISS, integrated summary of safety; MRX, maralixibat

Biological Plausibility

FSV absorption depends on fat solubilization and absorption. The liver is a site of lipid metabolism and bile salt production, which is important for fat absorption in the intestines. In the absence of components of bile in cholestasis, lipid solubilization and absorption are adversely affected. For example, BAs form mixed micelles which are important for solubilization and intestinal absorption of FSVs, such as vitamin A, D, E, and K, thus maintaining normal cell and organ function (Werner et al. 2000-2013).

Cholestasis is associated with malabsorption of FSVs (Phillips et al. 2001). Similar to liver test abnormalities, FSV deficiencies are a component of cholestatic disease. Therefore, determining the relatedness of maralixibat exposure to FSV deficiencies is difficult in this study due to the paucity of controlled data. Long-term exposure to maralixibat could lead to depletion of the BA pool, reducing luminal BA content and altering absorption of FSVs. An effect of maralixibat on FSV absorption is evidenced by an elevated prothrombin time/partial thromboplastin time and bleeding due to vitamin K deficiency at high doses of maralixibat in rat toxicology studies (Section 7.1).

Conclusion

FSV deficiencies were present in most subjects in LUM001-304 and the ISS at baseline. Increased doses and FSV replacement were necessary in a majority of subjects on maralixibat. Higher doses of maralixibat were associated with an increased need for FSV replacement, such as vitamin D. Subjects in OL extension studies were maintained on the highest tolerated doses. Therefore, it is unclear if the greater need for FSV replacement at higher doses is reflective of a dose-response effect of maralixibat on FSV deficiency, or if it reflects the longer duration of follow-up time. Any conclusions from these observations are therefore, limited by the absence of placebo-control.

7.7.4. Limited Placebo-Control Safety Database

Issue

LUM001-304 included a 4-week, randomized placebo-controlled withdrawal period after 18 weeks of OL treatment. This was the only placebo-controlled period comparing safety events of 400 mcg/kg/day maralixibat with placebo. Potential safety concerns associated with intermediate to long-term exposure are unlikely to be adequately evaluated in a 4-week placebo period. In addition, short-term safety signals (e.g., GI symptoms) may have been masked by the 18-week OL initial treatment period.

Background

The two supportive placebo-controlled randomized studies (LUM001-301 and -302) were 13-week placebo-controlled studies involving maralixibat doses of 70 to 280 mcg/kg/day. The placebo-controlled period in LUM001-304 was an RWD period after 18 weeks of OL exposure. Although the RWD period was adequate to demonstrate a benefit on pruritus, it was not adequate to fully evaluate potential short- and long-term safety signals.

Assessment

Gastrointestinal (GI) AEs are more likely to occur early in the course of treatment based on the mechanism of action of the drug (bile salt-induced diarrhea). Early onset of GI events were demonstrated both in LUM001-304 and in the supportive studies (Table 39 and Table 40). The protocols included gradual dose-escalation over a 5- or 6-week period to minimize drug discontinuation from GI AEs. Although the supportive studies were 13-week placebo-controlled studies, the doses tested were lower doses than in the pivotal study LUM001-304 and may not reflect the potential for GI AEs with the marketed dose of 400 mcg/kg. A total of 29 of 31 subjects entered the RWD period (2 discontinued for reasons unlikely to be related to study drug exposure during the 18-week OL period), leaving a comparison group of 16 placebo and 13 who remained on maralixibat. The 4-week RWD period was short, and it is possible there was some residual effect from prior treatment in the placebo arm during much of that period (e.g., the mean pruritus [ItchRo(Obs)]) score in the placebo arm did not approach baseline levels until Week 3 of the RWD period).

Table 39. Time to First Gastrointestinal Adverse Event In LUM001-304 (n=31)

Adverse Event (# Subjects with AE)	Diarrhea (N=17)	Abdominal Pain (N=19)	Vomiting (N=16)
Median time to onset (304)	36 days	43 days	84 days
Proportion occurring within open-label period (18 weeks)	13/17 (76%)	13/19 (68%)	11/16

Source: Reviewer Analysis of Adverse Events Data Set adae.xpt of ISS.
[1] Time to incident adverse event for each category
Abbreviation: AE, adverse event

Table 40. Time to First Gastrointestinal Adverse Event in the Drug Development Program (n=86)

Adverse Event (# Subjects with AE)	Diarrhea (N=48)	Abdominal Pain (N=46)	Vomiting (N=35)
Median time to first event	26 days	26 days	72 days
Proportion occurring within 18 weeks of treatment	38/48 (79%)	34/46 (74%)	23/35

Source: Reviewer Analysis of Adverse Events Data Set adae.xpt of ISS.
[1] Time to incident adverse event for each category
Abbreviation: AE, adverse event

Other AEs, such as liver test abnormalities, FSV deficiency, and bone fractures are known complications of ALGS, and any effects of maralixibat exposure may be more likely to manifest during longer exposure (Table 41). Therefore, long-term exposure without placebo control is unlikely to determine if maralixibat exposure affects the incidence or severity of these AEs.

Table 41. Time[1] to Adverse Events in the Drug Development Program (n=86)

Adverse Event (Number of Subjects with Event)	Days to First Event Median (Range)
Liver test abnormalities[1] (n=19)	342 (3-1583)
FSV Deficiency[2] (n=22)	232 (10-1756)
Bone fractures (n=8)	661 (118-2068)
GI Bleeding[2] (n=9)	403 (13-1696)

Source: Reviewer Analysis of Adverse Events Data Set adae.xpt of ISS.
[1] Time to incident adverse event for each category
[2] Terms were defined as follows:
Liver test abnormalities: ALT elevation and TB elevation
FSV deficiency: Vitamin D, A, E, K decrease, INR increase
GI Bleeding: Gastrointestinal hemorrhage, hematemesis, hematochezia, melena
Abbreviations: FSV, fat-soluble vitamin; GI, gastrointestinal

Conclusion

The safety database comprises limited placebo-controlled data, hampering evaluation of potential safety signals associated with short- or long-term exposure. This is made more difficult by the presence of these events in the natural history of ALGS, especially effects on FSV deficiency, bone fractures, abnormal liver enzymes, and growth and development. Maralixibat exposure may contribute to these outcomes, based on animal model data indicating a potential effect on FSV deficiency and healthy adult volunteer data demonstrating ALT elevation. The rarity of ALGS and the morbidity of severe pruritus symptoms make it impracticable to carry out long-term placebo-controlled studies to fully evaluate these potential signals. Postmarketing studies are planned to further assess long-term safety.

8. Therapeutic Individualization

8.1. Intrinsic Factors

Age and Weight

The Applicant has collected maralixibat concentration data in adolescents and children aged 10 to 17 years with primary hypercholesterolemia in Study NB4-02-06-014. Further, plasma drug concentrations were evaluated in pediatric participants aged 1 to 17 years in studies LUM001-301, LUM001-302, LUM001-303, LUM001-304, LUM001-305, and LUM001-501. The effect of age or body weight on PK of maralixibat could not be fully evaluated due to the limited PK data. In healthy adults, maralixibat was not measurable at doses lower than 20 mg (equivalent to

286 mcg/kg for a 70 kg). In pediatric patients with ALGS, maralixibat levels were below the limit of quantitation in the sparse PK samples that were collected. Based on the minimal systemic absorption and the proposed body weight-based dosing, significant effect of age or body weight on the systemic exposure to maralixibat is not expected.

Hepatic Impairment

The safety and efficacy of maralixibat in patients with portal hypertension and decompensated cirrhosis has not been studied. No dedicated PK study in patients with cirrhosis (hepatic impairment) was conducted. Patients with decompensated liver disease were excluded from the studies. Because maralixibat acts locally in the ileum and has low systemic absorption, the potential change in systemic exposure to maralixibat due to hepatic impairment is not expected to be clinically significant. Nevertheless, additional monitoring for AEs in patients with hepatic impairment should be recommended.

Renal Impairment

Maralixibat has not been studied in individuals with renal impairment. Given the limited systemic absorption and negligible renal excretion of maralixibat (<0.01% of the administered dose) in the mass balance study (NB4-02-06-004), it is unlikely that renal impairment would have a clinically relevant effect on the systemic exposure to maralixibat. As a result, dose adjustment for subjects with renal impairment is not necessary.

8.2. Drug Interactions

Bile Acid-Binding Resins

The Applicant has not conducted a systematic assessment of the effects of BA-binding resins on the efficacy of maralixibat. In the pivotal study LUM001-304, administration of BA-binding resins was prohibited due to potential influences on the efficacy assessment. It is plausible that BA-binding resins (e.g., cholestyramine, colesevelam, colestipol) bind maralixibat in the gut if given concurrently, potentially reducing maralixibat efficacy.

As a result, maralixibat should be taken at least 4 h before or after taking a BA-binding resin.

Drug-Metabolizing Enzymes and Drug Transporters

Since maralixibat is minimally absorbed, the potential for systemic drug interactions with other drugs is unlikely. However, drug interactions in the GI tract are plausible. The Applicant conducted three clinical drug-drug interaction (DDI) studies of the effect of maralixibat on the PK and/or pharmacodynamics of lovastatin, simvastatin, and atorvastatin (substrates of organic anion transporting polypeptide [OATP]2B1 and cytochrome P450 [CYP]3A4) (Study NB4-02-06-008 in hypercholesterolemic subjects and Studies NB4-01-06-019 and NB4-02-06-020 in healthy subjects). Refer to Section III.14.2.4 for details of the study design.

Maralixibat inhibits CYP3A4 in vitro; however, considering the minimal absorption into systemic circulation and the projected concentrations in the gut, clinically relevant effects on the PK of CYP3A4 substrates are predicted to be minimal. Using the static model, the change in AUC ratio was predicted to be less than two-fold at the highest dose. Maralixibat inhibits the drug transporter OATP2B1 in vitro, which may result in reduced absorption of drugs that rely on

OATP2B1-mediated uptake from the GI tract. The effect of maralixibat on the PK and/or pharmacodynamics of lovastatin, simvastatin, and atorvastatin (substrates of OATP2B1 and CYP3A4) was assessed in three clinical drug-drug interaction studies (Study NB4-02-06-008 in hypercholesterolemic subjects and Studies NB4-01-06-019 and NB4-02-06-020 in healthy subjects). Results indicated that co-administration of 20 mg lovastatin, simvastatin, or atorvastatin with or without a 10 to 12 h stagger, does not increase systemic exposure to maralixibat. In addition, coadministration of 4.75 mg of maralixibat (once daily) with daily doses of simvastatin, lovastatin, or atorvastatin did not have a clinically relevant effect on the PK of these statins and their metabolites.

However, the effect of maralixibat on the PK of OATP2B1 substrates has been evaluated only at low doses (≤5 mg); the effects of higher doses have not been assessed in a clinical study. As a result, reduced absorption of drugs that rely on OATP2B1-mediated uptake in the GI tract cannot be ruled out.

8.3. Plans for Pediatric Drug Development

Not applicable; this program was developed in all pediatric populations affected by pruritis in patients with ALGS.

8.4. Pregnancy and Lactation

Animal Data

The nonclinical information in Table 42 was used in support of the indicated labeling sections. Additional nonclinical data are located in Sections III.13.1 and III.13.2, and the final labeling is discussed in Section III.21.

Table 42. Nonclinical Data Supporting Labeling on Pregnancy and Lactation

Labeling Section	Nonclinical Data
8.1 Pregnancy	No effects on embryo-fetal development were observed in pregnant rats treated orally with up to 1000 mg/kg/day (approximately 3367 to 12,547-fold the maximum recommended dose based on AUC) or in pregnant rabbits treated orally with up to 250 mg/kg/day (approximately 1277 to 4758-fold the maximum recommended dose based on AUC) during the period of organogenesis. No effects on postnatal development were observed in a pre- and postnatal development study, in which female rats were treated orally with up to 750 mg/kg/day during organogenesis through lactation.
8.2 Lactation	Although the pre- and post-natal development study in rats produced clear evidence of maralixibat excretion in milk, the relevant data were not included in the label as explained below, under Lactation.

Source: Review team.
Abbreviation: AUC, area under the curve

Pregnancy

Maralixibat has low absorption following oral administration, and maternal use at the proposed clinical dose is not expected to result in measurable fetal exposure to maralixibat. There are no data on the use of maralixibat in humans during pregnancy. In animal reproduction studies, oral administration of maralixibat had no effect on embryo-fetal development (rats and rabbits) or postnatal development (rats). The dose levels used in the animal reproduction studies provided

extremely high multiples of the human AUC at the maximum recommended dose (i.e., approximately 3367- to 12547-fold in pregnant rats and 1277- to 4758-fold in pregnant rabbits).

Based on its mechanism of action, maralixibat may inhibit the absorption of dietary fats and lipid-soluble vitamins as well as bile salts. ALGS is often associated with malnutrition, and FSV supplementation is part of the standard of care. FSVs are necessary for normal development in a growing fetus, and maralixibat use may add further insult to already depleted FSV store in a pregnant patient with ALGS. FSV depletion in pregnancy may be detrimental to a growing fetus; therefore, based on discussion with the Division of Hepatology and Nutrition Clinical Team, the Division of Pediatric and Maternal Health (DPMH) agrees with adding language about monitoring for FSV deficiency in pregnant patients and supplementing FSVs if needed under Section 8.1.

Clinical Considerations

Alagille syndrome is a rare disease with an incidence of 1 in 30,000 to 1 in 70,000. Only 11 successful pregnancies have been reported to date. Additionally, systemic absorption following oral administration of maralixibat is low, and maternal use is not expected to result in fetal exposure. Therefore, DPMH does not recommend a postmarketing pregnancy study at this time.

Lactation

Maralixibat has low absorption following oral administration, and breastfeeding is not expected to result in measurable exposure of the infant to maralixibat at the clinical dose. There are no data on the presence of maralixibat in human milk, the effects of the drug on the breastfed infant, or on milk production.

In a pre- and post-natal developmental study of maralixibat in rats, there were no adverse effects on postnatal development. However, maralixibat was detected in rat pup plasma at all tested doses, which was likely due to transfer from rat milk rather than prenatal exposure. The lactating rats were given doses of maralixibat at human exposure (AUC) multiples that were 257- to 956-fold the maximum recommended human dose of 28.5 mg per day. Therefore, the clinical significance of this finding is uncertain.

Because there is low absorption of the drug after oral administration, breastfeeding is not expected to result in significant exposure of the infant to Livmarli (maralixibat chloride). Therefore, DPMH does not recommend a lactation study at this time.

Females and Males of Reproductive Potential

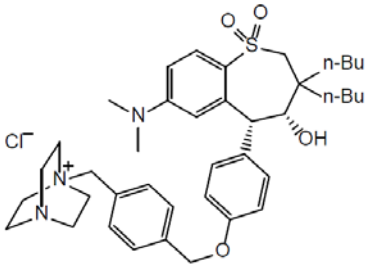
There are no reports of maralixibat adversely effecting human fertility. A study in rats showed no effects on mating and fertility in males or females of reproductive potential. There are no data to suggest maralixibat interacts with systemic hormonal contraceptives. DPMH recommends omitting subsection 8.3.

9. Product Quality

The drug substance in Livmarli is maralixibat chloride. It is a white to yellow solid. It is a chiral molecule containing two chiral centers. Two crystalline polymorphs (b) (4) have been identified but the drug substance is prepared (b) (4) It is soluble in water and sparingly

soluble in alcohol. Its chemical structure, molecular formula, and molecular weight are shown in Figure 13.

Figure 13. Chemical Structure of Maralixibat



Source: Applicant's Figure in Section 3.2.S.1.2 Structure of NDA Submission January 29, 2021
Molecular formula: $C_{40}H_{56}ClN_3O_4S$
Molecular weight: 710.42 g/mol

Maralixibat chloride is manufactured, tested, and packaged in accordance with current Good Manufacturing Practices at (b) (4). The chemistry, manufacturing, and controls information regarding the raw materials, intermediates, drug substance manufacturing process, critical steps, and in-process controls is deemed adequate. The potential genotoxic impurities are controlled in the drug substance specification per International Council for Harmonization M7 guidelines.

The chemical structure of maralixibat chloride was determined by elemental analysis, proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectroscopy; infrared spectroscopy, ultraviolet spectroscopy, mass spectrometry, single crystal X-ray crystallography, and X-ray powder diffraction.

Based on the results of long-term and accelerated stability testing of registration and supporting batches of drug substance, a retest period of (b) (4) months is granted when stored at controlled room temperature with excursions permitted between (b) (4) °C in the proposed container-closure system.

Livmarli (maralixibat) oral solution, 9.5 mg/mL is a clear, colorless to yellow, grape-flavored, nonsterile liquid. Each milliliter of Livmarli contains 10 mg of maralixibat chloride. It is supplied in multidose 30 mL round, amber plastic bottles. Calibrated 0.5, 1, or 3 mL dosing dispensers will be provided by the pharmacy. The oral solution contains the following inactive ingredients: edetate disodium, grape flavor, propylene glycol, purified water, and sucralose.

Livmarli oral solution is manufactured, tested, and packaged by (b) (4). The overall control strategy for the drug product's identity, strength, purity, and quality is deemed adequate based on raw material controls, manufacturing process, in-process controls, and drug product specification. The drug product specification includes microbial enumeration testing for total aerobic microbial count and total combined yeast/mold count per United States Pharmacopeia <61> and for the specified microorganisms *Escherichia coli* per United States Pharmacopeia <62> and *Burkholderia cepacia* complex per United States Pharmacopeia <60>.

Based on the satisfactory long-term and accelerated stability data of the drug product, a 24-month expiration dating period from the date of bulk solution manufacturing is granted when

the drug product is stored at 20°C to 25°C (68°F to 77°F) with excursions permitted to 15°C to 30°C (59°F to 86°F).

In the amendment dated August 26, 2021 the applicant committed to submit the drug product

container closure system leachables data of 24-month long-term stability samples by August 31, 2022 via a CBE-0 supplement.

In the amendment dated September 17, 2021 the applicant committed to provide an

extractables or leachables study report via a CBE-0 supplement by Oct 31, 2021 in response to the PMC from the Office of Pharmaceutical Manufacturing and Quality Assessment (OPMA).

The Applicant has provided sufficient chemistry, manufacturing, and controls information to assure the identity, strength, purity, and quality of the proposed Livmarli (maralixibat) oral solution.

The Office of Pharmaceutical Manufacturing Assessment has made a final overall Approval recommendation for the facilities involved in this application.

The claim for the Categorical Exclusion for the Environmental Assessment is granted.

The labeling is acceptable from the CMC perspective.

Therefore, from the Office of Pharmaceutical Quality perspective, this NDA is recommended for Approval from the OPQ product quality perspective.

9.1. Device or Combination Product Considerations

Not applicable.

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure

The Applicant stated that “This study was performed in compliance with Good Clinical Practices and applicable regulatory requirements, including the archiving of essential documents.”

These practices included Institutional Review Board/Independent Ethics Committee procedures, informed consent, protocol adherence, administrative documents, drug supply accountability, data collection, patient records (source documents), AE recording and reporting, inspection and audit preparation, and record retention. The investigator was made aware that regulatory authorities and representatives of the Applicant could inspect the documents and patient records at any time. All patient identities were kept confidential. Each patient was assigned a unique patient number, which in turn was used in the electronic case report form instead of the patient’s name.

The Office of Scientific Investigations inspected three clinical sites that participated in Study LUM001-304, as well as Mirum Pharmaceutical, Inc. There were no concerns identified and the efficacy and safety data in this NDA appear to be reliable.

Financial Disclosures

See Section III.23.

11. Advisory Committee Summary

An Advisory Committee meeting was not held for this NDA because the evaluation of the application did not raise significant safety or efficacy issues that were unexpected in the indicated population.

III. Appendices

12. Summary of Regulatory History

Table 43. Summary of Regulatory History

Date	Activity	Outcome
September 11, 2012	Pre-IND meeting	(b) (4)
October 24, 2013	IND 119917 submitted for the treatment of ALGS	November 22, 2013 IND 119917 was deemed Safe to proceed.
(b) (4)		
October 24, 2016	Guidance meeting	Formal meeting to discuss the Phase 3 drug development needed for marketing approval.
November 19, 2019	Pre-NDA Meeting	Discussion on the adequacy of the efficacy and safety data and additional data and analyses. In addition, there were discussions on PK and PD dosing. FDA clarified that the dose-response relationship should be explored for serum bile acid PD marker in the target patient population to justify dosing rationale. Further, FDA requested additional data for all COA endpoints based on the ItchRO.
December 2, 2019	Rare Pediatric Disease Designation	Granted.
April 6, 2020	Proprietary Name Review Request	Review of the proposed proprietary name, Livmarli, was conditionally acceptable.

Date	Activity	Outcome
May 21, 2020	Pre-NDA Clinical Meeting	Pre-NDA Clinical Meeting was requested January 30, 2020, granted on February 11, 2020, and written responses issued on May 21, 2020. FDA provided detailed comments to datasets and data standards in their proposed safety analysis strategy for the Integrated Summary of Safety The Division recommended the Sponsor submit a detailed analysis plan for the Integrated Summary of Safety (ISS) incorporating specific elements as described in the November 19, 2019 pre-NDA meeting min, as well as a pooling strategy, specific queries including use of specific standardized MedDRA queries (SMQs), and other important analyses intended to support safety.
September 11, 2020	Pre-NDA CMC Meeting	Pre-NDA CMC Meeting was requested on June 29, 2020, granted on July 21, 2020, and written responses issued on September 11, 2020. Mirum submitted the CMC Module, received August 31, 2020, the first section of their rolling review. Detailed comments and referenced guidances relevant CDER Pharmaceutical quality and ICH Quality Guidelines. The actual protocols, acceptance criteria, and study outcomes (as applicable) will be evaluated during an inspection of your manufacturing facilities. The product design and the suitability of manufacturing processes and control strategy will be evaluated during the NDA review cycle. FDA reminded Mirum that the Agency does not approve process validation approaches, protocols, or number of specific batches used in process validation studies; these will be reviewed during the manufacturing facilities inspection. It is Mirum's responsibility to conduct all studies necessary to assure your commercial manufacturing process is capable of consistently delivering quality product.

Source: Reviewer generated table.
Abbreviations: ALGS, Alagille syndrome; CDER, Center for Drug Evaluation and Research; CMC, chemistry, manufacturing, and controls; COA, clinical outcome assessment; FDA, Food and Drug Administration; FICI, fractional inhibitory concentration indices; ICH, International Council on Harmonisation; IND, investigational new drug; ItchRO, Itch Reported Outcome; MedDRA, Medical Dictionary for Regulatory Activities; NDA, new drug application; PBC, primary biliary cirrhosis; PD, pharmacodynamics; PFIC, progressive familial intrahepatic cholestasis; PK, pharmacokinetics; PSC, primary sclerosing cholangitis

There were no further meetings, and Mirum did not have communications with FDA regarding endpoint selection, study design, and Clinical Outcome Assessment instruments prior to data unblinding.

On August 31, 2020, Mirum submitted the first module (Administrative Documents, Literature References, and Summary of Nonclinical) of the rolling NDA submission.

The November 12, 2020 submission included Administrative Documents, Module 2 Summaries, Modules 3, 4, and 5 (clinical and nonclinical study reports), Request for Proprietary Name Review, and Draft Labeling.

On January 29, 2021, Mirum submitted the final modules (chemistry, manufacturing, and controls and additional nonclinical summaries) for this new molecular entity, new drug application (NDA); the Applicant requested for a priority review. The review time clock started on this date because all modules of the NDA were submitted. The proposed indication was the treatment of pruritus in pediatric patients (≥1 year of age) with Alagille syndrome (ALGS). Mirum requested an official waiver of the need to conduct a formal thorough QT study for maralixibat. Mirum submitted the proposed Prescribing Information (PI), Instructions For Use, and Patient Information.

13. Pharmacology Toxicology: Additional Information and Assessment

13.1. Summary Review of Studies Submitted Under the IND

13.1.1. Pharmacology

The pharmacological activity of SD-5613 (LUM001, maralixibat) was studied in vitro and in vivo. The studies were submitted in investigational new drug (IND) (b) (4), and 119917.

13.1.1.1. Primary Pharmacology

A baby hamster kidney cell line (H-14) that constitutively expresses human recombinant ASBT was used to measure the uptake of [^{14}C]taurocholate. In baby hamster kidney cells, maralixibat was shown to be a potent, competitive, and reversible inhibitor of the uptake of [^{14}C]taurocholate by the ASBT (IC_{50} 0.28±0.03nM). The K_m for taurocholate was 30µM. Maralixibat also inhibited [^{14}C]alanine uptake via another cellular sodium-dependent cotransporter; however, the potency was markedly lower (IC_{50} 35,700±3000nM). In transfected Chinese hamster ovary cells, maralixibat at 50µM produced a 5% inhibition of liver sodium taurocholate cotransporting polypeptide activity. Sodium taurocholate cotransporting polypeptide is 35% homologous to human ASBT and mediates uptake of bile salts into hepatocytes.

In vivo studies with maralixibat were conducted in rats, dogs, and monkeys, using single or repeated oral dosing (gavage or capsules) of up to 20 mg/kg for up to 4 weeks. Maralixibat produced a dose-dependent increase in fecal bile acid (fBA) excretion, inhibition of the postprandial rise in serum bile acids (sBAs) (dogs), and a decrease in total serum cholesterol. The in vivo pharmacology studies are summarized in Table 44.

Table 44. Summaries of In Vivo Primary Pharmacology Studies

Study Title/ Number	Route/Species	Dose Range	Key Findings
In vivo efficacy of SD-5613 in a rat gavage model (b) (4)98D1920)	Oral gavage Male Wistar rats	0.0006-2 mg/kg QD × 4 days	SD-5613 produced a dose-dependent increase in fBA excretion (1.2- to 3.6-fold compared to controls) with an ED_{50} of 0.027 mg/kg.
Dose form comparison of SD-5613 in dogs (b) (4)98Dl921)	Oral Beagle dog	1 and 4 mg/kg (powder in capsule) 1 and 4 mg/kg (solution in capsule) QD × 14 days	SD-5613 in both dose forms significantly increased fBA excretion in a dose-dependent manner by approximately 3- to 7-fold compared to pretreatment values. Serum cholesterol (total) and HDL cholesterol were reduced by 13-26% and 10-20%, respectively. Dose form had no effects on pharmacological activity.
Comparative effects of SD-5613, SC-77347 (solution and powder) and SC-78433 in normal-feed dogs (b) (4)99D1945)*	Oral Beagle dog	1 and 4 mg/kg QD × 7 days	SD-5613 produced a dose-dependent and significant increase in fBA excretion (200% and 303% at 1 and 4 mg/kg, respectively) and a significant decrease in serum cholesterol (total).
Efficacy of SD-5613: temporal dependence of dosing, relative to feeding (b) (4)99D1946)	Oral Beagle dog	Oral capsule 2 mg/kg QD × 7 days (dose was given 30 min or 4 h prior to feeding, or 4 h after feeding)	When administered 30 min before or 4 h after a meal, SD-5613 produced a significant increase in fecal bile acid excretion, approximately 230% compared to pretreatment values. A 67% increase in fecal bile acid excretion was observed with dosing of SD-5613 at 4 h before a meal.
SD-5613: in vivo efficacy in dogs (b) (4)98D1919)	Oral gavage or oral capsule Beagle dog	0, 1, 2, and 4 mg/kg (oral gavage) 2 mg/kg (oral capsule) QD × 7 days	SD-5613 administered by oral gavage at doses of 2 and 4 mg/kg produced a dose-dependent increase in fBA excretion (69% and 131%, respectively). A similar increase was observed with 2 mg/kg via oral capsule. SD-5613 had no effects on serum cholesterol (total), HDL cholesterol, or triglyceride levels.
SD-5613: effects on fBA excretion in cynomolgus monkeys (b) (4)98Dl923)	Cynomolgus monkeys Oral gavage	1.0, 2.5, 5, and 20 mg/kg QD × 7 days	SD-5613 produced a dose-dependent and significant increase in fBA excretion at 5 and 20 mg/kg/ day, compared to pretreatment values (2.4- and 5.4-fold, respectively).

Study Title/ Number	Route/Species	Dose Range	Key Findings
SD-5613 form I, SD-5613 form II, SC-78433, Glaxo-Welcome GW-264 and Cholestyramine: inhibition of postprandial rise in total sBA in dogs* (b) (4)98Dl927)	Oral capsule Beagle dog	0.02, 0.05, 0.2, 0.6, 2, 5, and 15 mg/kg (solution in capsule) 0.05, 0.2, and 0.6 mg/kg (powder in capsule); Single dose	Both dosage forms of SD-5613 were equally potent. SD-5613 at 0.05, 0.2, and 0.6 mg/kg solution in capsule or powder in capsule inhibited the postprandial rise in sBA in the range of 25.4% to 65.0% and 30.0% to 79.2%, respectively. The ED_{50} for solution in capsule dosage form was 0.2 mg/kg.

S : Prepared by the nonclinical reviewer.
SD-5613: code name for maralixibat
*Summary of results is limited to maralixibat (SD-5613).
Abbreviations: ED_{50}, 50% effective dose; fBA, fecal bile acids; HDL, high-density lipoprotein; QD, once per day; sBA, serum bile acids

13.1.1.2. Safety Pharmacology

Safety pharmacology studies in rats (neurobehavioral; Study #EX4879), dogs (cardiovascular; Study #4887 and 4898), and guinea pigs (pulmonary function; Study #4894) showed no significant changes following a single oral dose (up to 150 mg/kg) or intravenous dose (up to 1.9 mg/kg) of maralixibat. See Table 45.

Table 45. Summary of Safety Pharmacology Studies

Study/Study No.	Findings
Four-Day Neurobehavioral Study of Intravenous and Orally Administered SD-5613 in Rat/Study No. EX4879 Species/strain: CD non-IGS albino rats Number/group: 6/sex Doses: Oral: 150 mg/kg/day for 4 days IV bolus injection: 0.15 and 0.3 mg/kg/day for 4 days Route of administration and dosing frequency: oral and IV, once daily for 4 days	No effects on neurobehavioral function in rats were observed with either oral or intravenous administration of SD-5613 for 4 days.
Evaluation of Hemodynamics and Electrocardiograms in Conscious Beagle Dogs after Oral Administration of SD-5613/Study No. EX4898 Species/strain: Beagle dogs Number/group: 4 males Doses: 2, 6, 20 mg/kg Route of administration and dosing frequency: oral capsule, single dose	No effects on hemodynamics or ECG occurred in conscious beagle dogs treated with single oral doses of SD-5613, up to 20 mg/kg.
Acute Hemodynamic Effects of the Intravenous Administration of SD-5613 in Anesthetized Beagle Dogs/Study No. EX4887 Species/strain: Beagle dogs Number/group: 2 males in control group and 4 males in treatment group Dose: A loading dose (0.28 mg/kg) of SD-5613 was given as a bolus IV injection, followed by a maintenance dose infusion (0.50 mg/kg/45 min) for 45 min Route of administration and dosing frequency: IV, single dose	No effects on hemodynamic or electrocardiographic parameters were observed in anesthetized beagle dogs treated with a single IV dose of 0.78 mg/kg SD-5613.
Pulmonary Function Study of SD-5613 in the Guinea Pig/Study No. EX4894 Species/strain: Guinea pig Number/group: 5 males Dose: one group received a loading dose of SD-5613 (0.39 mg/kg) as a bolus intravenous injection; one group of animals received a maintenance IV infusion (1.9 mg/kg/45 min) for 45 min Route of administration and dosing frequency: IV, single dose	SD-5613 at an IV dose of 1.9 mg/kg had no significant effect on respiratory functions in guinea pigs.

Source: Prepared by the nonclinical reviewer.
SD-5613: code name for maralixibat
Abbreviations: ECG, electrocardiogram; IV, intravenous

13.1.2. Absorption, Distribution, Metabolism, Excretion / Pharmacokinetics

13.1.2.1. Distribution and Excretion

Mass Balance of [^{3}H]Maralixibat after Oral Administration in the Beagle Dog (Study No. M2098132)

Three female dogs received a single dose of [^{3}H]maralixibat (7.5 mg free base/kg), via oral gavage. Blood samples were collected predose and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 24, 48, 72, 96, and 120 h postdose. Urine and feces samples were collected predose (approximately -24 to 0 h) and at 24 h intervals up to 168 h postdose. At the time of sacrifice, the stomach, duodenum,

jejunum, ileum, and colon were collected. Total radioactivity in the samples was analyzed by liquid scintillation counting (LSC).

The concentrations of total radioactivity in plasma were low at all time points and declined rapidly after dosing. The mean maximum concentration (C_{max}) and time to maximum concentration (T_{max}) were 33.8 ng equivalent/mL and 0.92 h, respectively. The mean area under the concentration-time curve from time 0 to 8 h and area under the concentration-time curve from time 0 to 24 h (AUC_{0-24h}) were 151 and 336 ng equivalent•h/mL, respectively.

Concentrations of total radioactivity in cellular fractions were very low and below the limit of detection (8.40 ng equivalent/g) by 1.5 h postdose. Radioactivity concentrations in the cellular fraction were lower than the corresponding concentrations in plasma at all time points.

The percentage of total radioactivity in collected tissues was <0.005% of the total dose. The mean concentrations of radioactivity in the stomach, ileum, and jejunum were 21.4, 3.79, and 1.38 ng equivalent/g, respectively. The duodenum and colon radioactivity levels were below the limit of detection of 4.20 ng equivalent/g.

13.1.3. Toxicology

13.1.3.1. General Toxicology

SD-5613 [Maralixibat]: Twenty-Six-Week Dietary Toxicity Study in Rats with a Four-Week Reversal (SA4988/MSE-N 99078/R7244MSHP625)

Key Study Findings

- Maralixibat at 750 or 2000 mg/kg/day caused deaths due to fatal bleeding. Deaths were directly attributed to vitamin K deficiency, which resulted from the pharmacological activity of maralixibat (i.e., impaired absorption of fat-soluble vitamins (FSVs) due to loss of bile acids [BAs]).
- Significant decreases in body weight and body-weight gain in all males and mid- and high-dose females.
- Significant decrease in platelet volume in the mid- and high-dose groups.
- Significant increases in prothrombin time and activated partial thromboplastin time (aPTT) in all males and high-dose females, which was related to vitamin K deficiency due to the pharmacological activity of the drug.
- Significant increase in ALT in all males and high-dose females.
- Significant increase in AST in the high-dose groups.
- Decreased sBAs and increased fBAs, which were related to the pharmacological activity of the drug.
- Decrease in FSV (A, D, and E) levels in serum and liver.
- Significant increases in serum phosphorus and urinary calcium excretion in the mid- and high-dose groups.
- Increased incidence and severity of mucous depletion of goblet cells together with edema in the mucosal/submucosal lamina propria of the cecum, colon, and rectum at all doses; increased incidence and severity of nonsuppurative inflammation of the mucosa/submucosa of the cecum at all doses. These changes were likely related to the

increased concentration of BAs (pharmacologic effect of the test article) in the lumen of the cecum, colon, and rectum of treated animals.

- No target organ of toxicity was identified.
- The no observable adverse effect level (NOAEL) in males was 150 mg/kg/day ($AUC_{0\text{-}24h}$ 108 ng•h/mL), based on mortality in the 750 mg/kg/day males. The NOAEL in females was 500 mg/kg/day ($AUC_{0\text{-}24h}$ 241 ng•h/mL), based on mortality in the 2000 mg/kg/day females.

Table 46. Information, Study SA4988/MSE-N 99078/R7244MSHP625

Study Features and Methods	**Details**
Conducting laboratory and location: GLP compliance: Yes	(b) (4)
Methods	
Dose and frequency of dosing:	Males: 0, 30, 150, 750 mg/kg/day Females: 0, 30, 500, 2000 mg/kg/day Ad libitum
Route of administration:	Oral (dietary)
Formulation/vehicle:	Test article was mixed in rodent diet
Species/strain:	Rats/Sprague-Dawley
Number/sex/group:	15 (main study groups) 10 (recovery groups, control and high dose)
Age:	6 weeks
Satellite groups/ unique design:	12/sex/group for toxicokinetics (TK)
Deviation from study protocol affecting interpretation of results:	The dose for surviving high-dose males was reduced from 750 to 300 mg/kg/day on Day 88. The 12 surviving TK male animals from group 5 (dosed at 150 mg/kg/day) were reassigned to recovery group 2 (dosed at 150 mg/kg/day) on Day 92. Dosage for surviving high-dose females (Group 3 and TK group 6) was reduced from 2000 mg/kg/day to 1500 mg/kg/day on Day 102. The eight surviving TK male animals from group 6 (dosed at 300 mg/kg/day) were reassigned to recovery group 3 on Day 170. The six surviving TK female animals from group 6 (dosed at 1500 mg/kg/day) were reassigned to recovery group 3 on Day 170. The 10 surviving TK female animals from group 5 (dosed at 500 mg/kg/day) were reassigned to recovery group 2 on Day 170. The deviations from the study protocol are not expected to have affected the outcome.

Source: Prepared by the nonclinical reviewer.

Table 47. Observations and Results, Study SA4988/MSE-N 99078/R7244MSHP625

Parameter	Major Findings
Mortality	SD-5613 produced mortality at 750 mg/kg/day in males and 2000 mg/kg/day in females. By Day 87, 15 of 37 males in the 750 mg/kg/day group had died or were sacrificed in moribund condition. The high dose was reduced to 300 mg/kg/day for males on Day 88. One additional high-dose male died on Day 89. By Day 95, 7 of 37 females in the 2000 mg/kg/day group had been sacrificed in moribund condition. The high dose for females was reduced to 1500 mg/kg/day on Day 102. The deaths were mainly attributed to internal hemorrhage due to vitamin K deficiency, which resulted from impaired absorption of fat-soluble vitamins due to bile-acid deficiency. Intestinal gaseous distension and/or impaction was a minor cause of death. The Applicant stated that these effects were attributed to changes in the intestinal microenvironment (content and microbial flora) associated with the administration of a poorly absorbed test article and/or the increased levels of fecal bile acids.
Clinical signs	Paleness of skin and mucous membranes in the high-dose males. Focal loss of hair in the high-dose females. Reddish brown staining of the cage paper for animals in all treatment groups. Paleness of skin and mucous membranes was considered secondary to hemorrhage. The cause for reddish brown staining of cage paper is unknown, but repeated urinalyses excluded urinary blood.
Body weight/food consumption	Statistically significant decreases (85-90% of control values) in cumulative weight gain and group mean body weights in all male treatment groups and in the mid- and high-dose females. These changes were reversible. The changes in food consumption were not of toxicological significance.
Ophthalmoscopy	None
Electrocardiogram	Not applicable
Hematology	Significant decrease in mean platelet volume (MPV) in the mid- and high-dose male groups at Weeks 15 and 26 (93-96% of controls), mid-dose female group at Week 26 (95% of controls), and high-dose female group at Weeks 15 and 26 (95-96% of controls). Not reversible. Significant increases in prothrombin time and aPTT in males at all doses (115-199% of controls) without a clear dose effect, and in high-dose females (1.7-6.1-fold control values). Changes were reversible.

Parameter	Major Findings
Clinical chemistry	Significant increase in alanine aminotransferase in all male treatment groups (113-140% of control values) with no apparent dose-dependency, and in the high-dose females (107% of control values). Reversible (not observed in recovery groups). Significant increase in aspartate aminotransferase in males in the low- and high-dose groups (123-128% of control values) and in the high-dose females (127% of control values). Reversible. Significant decrease in high-density lipoprotein (HDL) in males at all doses (77-83% of control values) and in females in the high-dose group (81% of control value) with no apparent dose-effect. Not reversible in males. Significant decrease in triglycerides in males at all doses (55-75% of control values) and females in the high-dose group (56% of control values) with no apparent dose-effects (reversible). The changes in HDL and triglycerides were related to the pharmacological activity of the drug. Significant decreases in total protein (93-96% of control values), albumin (up to 94% of controls), and globulin (up to 88-90% of controls) in both sexes at all doses (reversible). Significant decrease in serum bile acids in all animals (47-90% of controls), which was related to pharmacological activity and was reversible. Significant increase in serum phosphorus levels in mid- and high-dose males (up to 119% of control values) and females (up to 118% of control values). Not reversible in males.
Urinalysis	Significant increase in urinary calcium excretion in the mid- and high-dose groups (up to 205% of control values in males and up to 177% of control values in females; reversible). Significant increase in urinary sodium excretion in the mid- and high-dose groups (up to 189% of control values in males and up to 216% of control values in females) with a dose-effect (reversible). These increases were attributed to cecal enlargement, which is associated with increased intestinal absorption and renal excretion of calcium.
Gross pathology	Drug-related macroscopic findings were observed only in animals found dead or sacrificed in moribund condition. Most of these animals had the following gross necropsy alterations: abnormal color (red/purple/black) in epididymis, lymph nodes, lung, pancreas, skeletal muscle, testis, thymus, stomach, and urinary bladder; general pallor of the carcass, abnormal contents (blood-like) in various hollow organs and body cavities. A few animals that died during the study had a distended large intestine (cecum and/or colon), and abnormal content and/or impaction in the cecum, ileum, small intestine, and/or stomach.
Organ weights	Dose-dependent and significant increases in absolute and relative cecum weights at all doses (107-186% of controls), except for the low-dose females. Significant increases in relative colon weight (111-119% of controls) at all doses except for low-dose females. Significant decreases in absolute and relative liver weights (78-89% of controls) in males at all doses. Increased cecum weight and decreased liver weight were not reversible.

Parameter	Major Findings
Histopathology Adequate battery: Yes	Microscopic findings in dead or moribund animals in the high-dose groups included: Hemorrhage and hemosiderin deposition in multiple tissues/organs (e.g., aorta, brain, duodenum, epididymis, eyes, heart, spinal cord, lung, lymph nodes, pancreas, pituitary, spleen, stomach, skeletal muscle, prostate, urinary bladder, thymus); erythroid hyperplasia in bone marrow of the femur and sternum; and fibrosis (with or without chronic inflammation) in the epididymis and pancreas. Drug-related changes that occurred at all doses were found in the cecum (minimal to mild submucosal edema, nonsuppurative inflammation of the mucosa/submucosa, minimal to moderate mucus depletion of goblet cells), rectum, and colon (minimal to moderate mucus depletion of goblet cells and lamina propria edema). The changes in the cecum were reversible. No data were provided for the colon and rectum at the end of the recovery period. All changes were likely related to the increased concentration of bile acids in the lumen of the cecum, colon, and rectum in treated animals (pharmacologic effect of the test article).
Fecal bile acids	Significant increases in fecal bile acids occurred at all doses (up to 641% of controls). This effect was related to the pharmacological activity of the drug and was not reversible in the high-dose group.
Serum and liver vitamins	Dose-dependent decreases in serum vitamin A, D, and E (20-94% of controls) and in liver levels of vitamin A and E (16-76% of controls) in females. Decreases in serum and liver levels of vitamin E in mid- and high-dose males (53-62% of controls). Increases in liver levels of vitamin A in mid- and high-dose males (53-62% of controls). Increases in liver levels of vitamin E in mid- and high-dose males at the end of the recovery period. Changes in serum vitamin A in the high-dose females and liver vitamin E in the mid- and high-dose females were not reversible. These effects were likely related to altered absorption of fat-soluble vitamins from the intestinal tract due to inhibition of bile acid reabsorption (i.e., depletion of bile acids).
Toxicokinetics	Blood samples were collected on Days 2 and 168 at 1200 h (12 PM), 1800 h (6 PM), 2400 h (12 AM), and 0600 h (6 AM). There were no consistent sex-related differences in plasma concentrations or TK parameters at 30 mg/kg/day. Sex-related differences could not be determined in the other dose groups because of differences in dose levels. The C_{max} and AUC values increased with increasing dose, generally in a less than dose-proportional manner. The toxicokinetic data are summarized in Table 48 and Table 49.

Source: Prepared by the nonclinical reviewer.
SD-5613: code name for maralixibat
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration

Table 48. Mean T_{max}, C_{max}, and $C_{max/dose}$ for Maralixibat in Male and Female Rats on Days 2 and 168

Dose (mg/kg/day)	T_{max} [a] (h)		C_{max} (ng/mL)		C_{max}/Dose	
	Male	Female	Male	Female	Male	Female
Study Day 2						
30 [b]	0600 [c]	0600 [c]	2.15 ± 1.24	2.71 ± 1.25	0.0715	0.0940
150	0600 [c]	-	11.2 ± 6.32	-	0.0745	-
500	-	0600 [c]	-	8.76 ± 3.06	-	0.0175
750	1200	-	17.4 ± 11.0	-	0.0233	-
2000	-	1200	-	59.1 ± 22.2	-	0.0296
Study Day 168						
30 [b]	1800	1800	2.60 ± 0.880	1.73 ± 0.305	0.0868	0.0576
150	1200	-	5.42 ± 1.65	-	0.0362	-
300	1200	-	14.8 ± 8.82	-	0.0493	-
500	-	1800	-	13.5 ± 5.29	-	0.0269
1500	-	0600 [c]	-	53.5 ± 8.58	-	0.0357

[b] Samples were collected at 1200, 1800, 2400, and 0600 h (the next day). For calculation purposes, collection times were assigned nomimal values of 6, 12, 18, and 24 h, respectively.
[b] Combined male and female mean (n=6) T_{max}, C_{max}, and C_{max}/Dose were 0600 [b] h, 2.43 ± 0.797 ng/mL, and 0.0810, respectively, on Day 2, and 1800 h, 2.17 ± 0.460 ng/mL, and 0.0722, respectively, on Day 168.
[c] This time point refers to blood sample taken on Day 3 or 169.

Source: Applicant's report SA4988/MSE-N 99078/R7244MSHP625.
Abbreviations: C_{max}, maximum concentration; T_{max}, time to maximum concentration

Table 49. Mean $AUC_{0\text{-}24h}$, $AUC_{0\text{-}24/dose}$, and Bioavailability of Maralixibat in Male and Female Rats on Days 2 and 168

Dose (mg/kg/day)	$AUC_{0\text{-}24h}$ (ng•h/mL)		$AUC_{0\text{-}24h}$/Dose		BA (%)	
	Male	Female	Male	Female	Male	Female
Study Day 2						
30 [a]	31.1	26.2	1.04	0.875	0.0703	0.0700
150	114	-	0.761	-	0.0514	-
500	-	132	-	0.263	-	0.0210
750	244	-	0.326	-	0.0220	-
2000	-	727	-	0.364	-	0.0291
Study Day 168						
30 [a]	20.8	28.3	0.692	0.944	0.0468	0.0755
150	108	-	0.723	-	0.0489	-
300	279	-	0.930	-	0.0628	-
500	-	241	-	0.481	-	0.0385
1500	-	940	-	0.626	-	0.0501

[a] Combined male and female mean (n=6) $AUC_{0\text{-}24h}$, AUC/Dose, and BA were 28.7 ng•h/mL, 0.955, and 0.0699%, respectively, on Day 2, and 24.5 ng•h/mL, 0.818, and 0.0599%, respectively, on Day 168.

Source: Applicant's report SA4988/MSE-N 99078/R7244MSHP625.
Abbreviations: $AUC_{0\text{-}24h}$, area under the curve from time 0 to 24 h; BA, bioavailability

SD-5613: A Combination Six-Month and One-Year Oral Capsule Toxicity Study in the Dog with Reversals (SA4987/D7243M-SHP625)

Key Study Findings

- Significant decrease in male body-weight gain at 20 and 100 mg/kg/day.
- Significant decrease in red cell indices in both sexes and increase in platelets in females at ≥5 mg/kg/day.
- Prolongation of prothrombin time at 100 mg/kg/day due to vitamin K deficiency, which resulted from the pharmacological activity of the drug (i.e., impaired absorption of FSVs due to loss of BAs).
- Significant increase in AST at all doses in females without dose-dependency.
- Pharmacological activity-related changes including significant decreases in cholesterol, high-density lipoprotein, and sBAs, and an increase in fBAs.
- Significant decreases in serum phosphorus (20 and 100 mg/kg/day females), calcium (all female treatment groups [≥1 mg/kg/day]), and ALP (all male treatment groups).
- Significant increases in urinary phosphorus excretion at all doses with dose-dependency, associated with decreased serum phosphorus and ALP, suggesting decreased osteoblastic activity.
- Decreased eosinophilia in parietal cells of fundic mucosa at 100 mg/kg/day and increased cytoplasmic vacuolation of pyloric mucosa in males at ≥5 mg/kg/day and in females at 5 and 100 mg/kg/day.
- The target organ of toxicity was the stomach.
- The NOAEL in both sexes was 5 mg/kg/day, with an $AUC_{0\text{-}24h}$ of 57.7 ng•h/mL on day 358. The NOAEL designation in males is primarily based on the nonreversible changes in the stomach, with additional consideration of the small decreases in bodyweight (6.9 to 7.8%) and body-weight gain (18 to 26%) at 20 and 100 mg/kg/day. The NOAEL designation in females is primarily based on the decreases in bodyweight (12.2 to 14.9%) and body-weight gain (47 to 54%) at 20 and 100 mg/kg/day.

Table 50. Information, Study MRXNC-004

Study Features and Methods	Details
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Methods	
Dose and frequency of dosing:	1, 5, 20, and 100 mg/kg/day; once daily
Route of administration:	Oral
Formulation/vehicle:	Gelatinous capsule
Species/strain:	Beagle dogs
Number/sex/group:	8-12
Age:	6.5-8.5 months
Satellite groups/ unique design:	4 animals/sex/group from control and 100 mg/kg/day groups were assigned to recovery groups. 4-week recovery period was allowed after the initial 6 months of treatment; 8-week recovery period was allowed after the 1 year of treatment.
Deviations from study protocol affecting interpretation of results:	None

Source: Prepared by the nonclinical reviewer.

Table 51. Observations and Results, Study MRXNC-004

Parameter	Major Findings
Mortality	None
Clinical signs	Emesis at all doses, with the incidence increased by approximately two-fold relative to the control group; pinkish fur discoloration at 100 mg/kg/day.
Body weights	Although no statistically significant changes in body weight were reported, the data show a clear dose-dependent decrease in body weight at Week 52 (up to 7.8% in males and 14.9% in females). Significant decrease in male body weight gain at 20 and 100 mg/kg/day (82% and 74% of controls, respectively at the end of the 1-year treatment). The weight gain in the control males was 3.07 kg, with a mean baseline weight of 9.43 kg. Nonsignificant decrease in body-weight gains in female groups at 5, 20, and 100 mg/kg/day (64%, 46%, and 53% of controls, respectively, at Week 52). The weight gain in control females was 2.70 kg, with a mean baseline weight of 7.43 kg. All changes were reversible.
Ophthalmoscopy	None
Electrocardiogram	None

Parameter	Major Findings
Hematology	Significant decreases in red blood cells (up to 11%), hemoglobin (up to 10%), and hematocrit (up to 10%) at ≥5 mg/kg/day at Weeks 13, 26, 39, and 51. The changes were reversible after the 4- and 8-week recovery periods. Significant increase in platelets in females at ≥ 5 mg/kg/day (up to 28%) at Weeks 13, 39, and 51 (reversible). Significant increase in prothrombin time (PT) at 100 mg/kg/day in males at Weeks 13, 26, 39, and 51 (6%, 5%, 12%, and 8%, respectively), and in females at Weeks 26, 39, and 51 (5%, 7%, and 5%, respectively). Reversible after 4 or 8 weeks of recovery. Significant decrease in fibrinogen at all doses at Weeks 13, 26, 39, and 51 (17-43%), dependent on dose and duration. The prolongation of PT is attributed to vitamin K deficiency due to pharmacologic activity (i.e., impaired fat-soluble vitamin deficiency due to reduced bile acid reabsorption).
Clinical chemistry	Significant increase in aspartate aminotransferase in females at all doses (up to 40% at Week 39 and 48% at Week 51), without dose dependency (irreversible). Significant decreases in cholesterol (up to 45%) and high-density lipoprotein (up to 48%) at all doses with a dose- and duration-dependent effect. The effects were related to pharmacologic activity. Decreases in triglycerides and serum total bile acids in all treated animals at Weeks 12 and 25. The levels were frequently below the level of detection. Significant decreases in total protein level (up to 11%) and globulin (up to 14%) at Weeks 13, 26, 39, and 51 at 100 mg/kg/day. Significant decrease in alkaline phosphatase (up to 54% at Week 39 and up to 57% at Week 51) in males at all doses without a clear dose-dependent effect. Significant decrease in phosphorus (up to 16%) in females at 20 and 100 mg/kg/day (irreversible). Significant decrease in calcium (up to 6%) at Week 51 in females at all doses.
Urinalysis	Significant increases in urinary phosphorus excretion in males at 100 mg/kg/day and in females at all doses at Week 12. Significant increases in urinary phosphorus excretion in males at 20 and 100 mg/kg/day and in females at 5, 20, and 100 mg/kg/day at Week 25. Significant, dose-dependent increase in urinary phosphorus excretion in males at 100 mg/kg/day (125.8%) and females at all doses at Week 38 (163-395%). Significant increase in urinary phosphorus excretion in males (101-118%) and females (279-326%) at ≥20 mg/kg/ day at Weeks 51-52. A dose-dependent increase in urinary phosphorus excretion correlated with decreases in serum inorganic phosphorus and serum alkaline phosphatase, suggesting decreased osteoblastic activity.
Gross pathology	None
Organ weights	None

Parameter	Major Findings
Histopathology Adequate battery: Yes	Decreased eosinophilia in parietal cells of fundic mucosa at 100 mg/kg/day on week 53 (all 4 males and 3/4 females); not reversible after 8-week recovery period. Cytoplasmic vacuolation of pyloric mucosa in males (1/4, 2/4, and 3/4 males in the 5, 20, and 100 mg/kg/day groups, respectively) and in females (1/4 and 3/4 females in the 5 and 100 mg/kg/day groups, respectively); not reversible.
Fecal bile acids (fBA)	Significant increase in fBA at all doses between Weeks 12 to 51 (up to 6.3-fold in males at Week 51, and 5.1-fold in females at Week 38; reversible).
Liver levels of vitamins A and E	Blood samples were collected at Weeks 26 and 51 for analysis of vitamins A, D, and E. Liver samples were collected at Weeks 27 and 53 and at the end of the recovery period (Week 61) for analysis of vitamins A and E. The data for vitamins A, E, and D in plasma and liver were not reliable due to major deficiencies in the analytical methods used by the initial testing laboratory. The amounts of vitamins A and E in liver at Week 53 and the end of the recovery period were analyzed by an alternative testing laboratory. SD-5613 at doses of 20 and 100 mg/kg/day produced dose-dependent decreases in the liver content of vitamin E (α-tocopherol; up to 47.4% in males and 41.9% in females) and vitamin A (up to 43.6% in males and 41.9% in females). There were no data for vitamin D. The decrease in vitamin A was irreversible.
Toxicokinetics	Blood samples for toxicokinetic (TK) analysis were collected at 1, 2, 3, 5, 8, and 24 h after dosing on Day 1 and during Weeks 26 and 52. At doses ≥5 mg/kg, SD-5613 was detected in a majority of the plasma samples. At the 1 mg/kg dose, the majority of plasma concentrations were below the assay sensitivity limit of 1 ng/mL. The bioavailability of SD-5613 was less than 1% at all doses on all sampling days. The C_{max} and AUC_{0-24h} were higher on Day 176 compared to Day 1 and were generally similar on Days 176 and 358. Exposure to SD-5613 increased with dose. The increase was less than dose-proportional on Day 1 and greater than dose-proportional on Days 176 and 358. The TK data are listed in Table 52.

Source: Prepared by the nonclinical reviewer.
Abbreviations: AUC_{0-24h}, area under the curve from time 0 to 24 h; C_{max}, maximum concentration; SD-5613, maralixibat

Table 52. Toxicokinetic Parameters in a 52-Week Oral Toxicity Study in Dog

Dose	Sex	C_{max} (ng/mL)			AUC (hr·ng/mL)		
		Day 1	Day 176	Day 358	Day 1	Day 176	Day 358
1	M and F	5.73 (0.903)	6.87	1.98	14.7 (2.61)	8.04	4.80
5	M and F	13.8 (2.55)	3.37	2.97	35.2 (6.90)	8.20	13.5 (13.6)
20	M and F	5.84	20.2	18.5	21.5	55.4	57.7
100	M and F	10.2	113	87.9	51.5	455	342

AUC = area under the curve; C_{max} = plasma concentration; F = female; M = male
Numbers in parentheses represent values obtained after the removal of outliers.
Source: Applicant's report SA4987/D7243M-SHP625.

13.1.3.2. General Toxicology: Additional Studies

Thirteen-Week Oral Gavage Toxicity Study of SD-5613 [Maralixibat] in Rat/Study No. *SA4947*

A 3-month oral toxicity study of maralixibat was conducted in Sprague–Dawley rats at dose of 0, 5, 30, 75, and 150 mg/kg/day in males, and 0, 5, 30, 150, and 500 mg/kg/day in females.

The bioavailability of the test article was <1% at these doses. maralixibat treatment produced a 4- to 8.7-fold increase in mean fBAs at doses ≥5 mg/kg, but the increase was not dose-, time-, or sex-dependent. Several animals in the maralixibat treatment groups died of gavage accidents and aspiration of the test article dosing solutions. All treatment groups showed increases in relative lung weights, associated with peribronchiolar histologic changes (e.g., alveolar histiocytosis with amphophilic amorphous material, accumulation of large foamy macrophages mixed with amorphous material and cholesterol clefts in the alveoli, and interstitial fibrosis with alveolar epithelialization). The changes in clinical pathology, organ weights, and histopathology associated with maralixibat treatment were mostly minimal to mild, reversible, and related to the pharmacologic activity or poor absorbability of the test article (e.g., mucus depletion of goblet cells, edema in the lamina propria of the colonic and rectal mucosa). There were no maralixibat-related findings considered adverse in this study. The NOAEL was 150 mg/kg/day in males and 500 mg/kg/day in females.

Thirteen-Week Dietary Toxicity Study of SD-5613 [Maralixibat] in Rat/Study No. SA5004 (SA5004/MSE-N00002/R7246M-SHP625)

A 3-month dietary toxicity study of maralixibat was conducted in Sprague–Dawley rat at dose levels of 0, 150, 750, and 1500 mg/kg/day. Deaths due to internal hemorrhages occurred in 1 male at 750 mg/kg/day and 18 males at 1500 mg/kg/day beginning on Day 17.

Maralixibatproduced significant increases in prothrombin time and aPTT (106 to 238% of control values) in males at 150 and 750 mg/kg/day, and increases in serum ALT and AST (111 to 148% of control values) in males at all doses. The decreases in total sBAs at 750 and 1500 mg/kg/day (40 to 85% of control) and an increase in fBAs in all test article groups (106 to 586% of control) were attributed to the pharmacologic action of maralixibat. Due to inadequate analytical methods, the results for serum and liver levels of vitamins A and E were invalid. Serum vitamin D concentrations were slightly reduced (74 to 83% of control) in the 750 and 1500 mg/kg/day groups at Week 14. However, there were no histological findings consistent with vitamin D deficiency. Absolute and relative liver weights were increased in males at the mid and high doses, whereas absolute and relative cecum weights were increased at all doses in both sexes. Microscopic changes were noted primarily in the distal colon (i.e., mucosal depletion of goblet cells and edema of the lamina propria), and the effects were more prominent at the low and mid doses. The increases in prothrombin time and aPTT in males may be indicative of vitamin K deficiency due to BA deficiency. The changes in coagulation and clinical chemistry were reversible. The increase in cecum weight was irreversible. The NOAEL was 150 mg/kg/day in males based on mortality at 750 mg/kg/day, and 750 mg/kg/day in females based on excessive loss of BAs and reduction in serum vitamin D at 1500 mg/kg/day.

SD-5613 [Maralixibat]: Thirteen-Week Gavage (Suspension) Study in Rat/Study No. MSE-N 00003/SA5006

A 3-month oral toxicity study of maralixibat was conducted in Sprague–Dawley rats using dose levels of 0, 150, 500, and 1500 mg/kg/day. All doses produced premature deaths. Most of the unscheduled deaths in males at 500 and 1500 mg/kg/day and in females at 1500 mg/kg/day were due to fatal bleeding events or gaseous distension in the intestinal tract. The prothrombin time and aPTT were increased in males at all doses and in females at 1500 mg/kg/day. These changes were considered to be adverse at 500 mg/kg/day in males and at 1500 mg/kg/day in females due to the associated fatal bleeding events. Prolongation of the prothrombin time and aPTT was likely due to vitamin K deficiency secondary to a drug-induced BA deficiency. Other clinical chemistry changes included dose-dependent increases in ALT and AST in males at all doses and in females at 1500 mg/kg/day and significant increases in alkaline phosphatase (ALP) in males at 1500 mg/kg/day and females at 500 and 1500 mg/kg/day. Pulmonary histologic changes in 15 animals that were found dead or sacrificed in extremis included fibrin deposition, inflammatory infiltrate, necrosis, and hemorrhage. No histopathological data were included in the study report, with the exception of pulmonary microscopic findings. The NOAEL was not identified due to drug-related deaths at all doses.

A 13-Week Oral Gavage Toxicity and Toxicokinetic Study with Maralixibat in Rat With a 4-Week Recovery/Study No. MRX-NC-004

A 3-month oral toxicity study of maralixibat was conducted in Sprague-Dawley rats using dose levels of 0, 10, 300, and 1000 mg/kg/day. The major findings included significant increases in food consumption at 1000 mg/kg/day, significant increases in AST and ALT in males at 10 and 1000 mg/kg/day, significant decreases in reticulocytes and fibrinogen in the 1000 mg/kg/day males, and a significant decrease in liver weight in the 10 and 1000 mg/kg/day males. The NOAEL was considered to be 1000 mg/kg/day due to the absence of adverse events.

SD-5613 [Maralixibat]: Thirteen-Week Oral Capsule Toxicity Study in the Dog/SA4991/7245M-SHP625

A 3-month oral toxicity study of maralixibat was conducted in beagle dogs using dose levels of 0, 5, 20, and 100 mg/kg/day. Maralixibat at 100 mg/kg/day produced clinical signs (emesis was increased by three-fold compared to the control group, from 38 to 151 incidences/week). Maralixibat at 100 mg/kg/day produced a significant increase in prothrombin time in males (8.1 versus 7.8 s in controls). A decrease in fibrinogen was observed in females at 20 and 100 mg/kg/day (128 and 137 mg/dL, respectively, compared to 194 mg/dL in the control group).

Treatment with 20 and 100 mg/kg/day maralixibat produced significant decreases in cholesterol and high-density lipoprotein and increases in sodium and chloride levels. Maralixibat produced significant increases in fBAs at all doses in males and at 100 mg/kg/day in females. No drug-related effects were observed in macroscopic or microscopic examinations. Maralixibat accumulated in plasma by two-fold at 100 mg/kg/day during the 3-month dosing period (503 versus 239 ng•h/mL on Day 1). Absolute bioavailability was <1%. The NOAEL is considered to be 20 mg/kg/day, based on the high incidence of emesis at 100 mg/kg/day.

13.1.3.3. Genetic Toxicology

Table 53. Genetic Toxicology Studies

Study No./Title	Key Study Findings
Study No. SA4883/ Evaluation of the Mutagenic Potential of SD-5613 in the Ames Salmonella/Microsome Assay GLP compliance: Yes Study is valid: Yes	Tester strains: TA97a, TA98, TA100, TA1535, and TA102. Highest dose: 1000 µg/plate Treatment duration: 2 days in the presence or absence of S9 metabolic activation SD-5613 was not mutagenic in this assay.
Study No. SA4884/ An Evaluation of the Potential of SD-5613 to Induce Chromosome Aberrations in Chinese Hamster Ovary Cells GLP compliance: Yes Study is valid: Yes	Highest dose: 70 µg/mL for 3 h and 45 µg/mL for 17.9 h without S9 metabolic activation; 110 µg/mL for 3 h with S9 metabolic activation Treatment duration: 3 and 17.9 h without metabolic activation and 3 h with metabolic activation SD-5613 did not induce chromosome aberrations under the test conditions.
Study No. SA4893/ An Evaluation of the Potential of SD-5613 to Induce Micronucleated Polychromatic Erythrocytes in the Bone Marrow of Rats GLP compliance: Yes Study is valid: Yes	Doses of 500, 1000, and 2000 mg/kg were administered to Sprague-Dawley rats by gavage. Animals were sacrificed at 24 and 48 h after dosing, and bone marrow cells were prepared for the evaluation of micronuclei in polychromatic or normochromatic erythrocytes. SD-5613 did not induce the formation of micronuclei in this assay.

Source: Prepared by the nonclinical reviewer.
SD-5613: code name for maralixibat
Abbreviations: GLP, Good Laboratory Practices

13.1.3.3.1. Reproductive and Developmental Toxicology

13.1.3.3.1.1. Fertility and Early Embryonic Development

SA5060/A Diet Admix Study of the Effects of Maralixibat On Fertility and Early Embryonic Development to Implantation in Male and Female Rats

Key Study Findings

- No effects on male and female mating or fertility indices.
- Reductions in the number of corpora lutea (per dam), number of implantation sites (per dam), and viable fetuses (per dam) occurred at 500 and 2000 mg/kg/ day. However, these changes were small in magnitude, and were likely due to mild toxicity rather than a direct effect of the test article.
- The NOEL for male reproductive toxicity was 750 mg/kg/day, and the NOAEL for female reproductive toxicity was 2000 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 54. Rat Oral Fertility and Early Embryonic Developmental Study Methods

Parameter	Method Details
Dose and frequency of dosing:	Males: 0, 30, 150, 750 mg/kg/day Females: 0, 30, 500, 2000 mg/kg/day
Route of administration:	Dietary (ad libitum)
Formulation/vehicle:	Not applicable.
Species/strain:	Sprague-Dawley rats (Crl:CD SD, IGS, BR)
Number/sex/group:	25
Satellite groups:	None
Study design:	Females were treated for 14 days prior to mating, throughout the mating period (with untreated males), and through gestation day 7. Mated females were sacrificed on day 15 of pregnancy. Males were treated for 28 days prior to mating and throughout the mating period (with untreated females), until their necropsy.
Deviation from study protocol affecting interpretation of results:	None

Source: Prepared by the nonclinical reviewer.

Table 55. Observations and Results

Parameter	Results
Mortality	One female at 2000 mg/kg/day and one male at 750 mg/kg/ day were found dead on Days 34 and 69, respectively. The cause of death was unknown.
Clinical signs	In females, treatment with 500 and 2000 mg/kg/day produced clinical signs (increased hair loss and scabbing on the forelimb, abdomen, and thorax).
Body weight/food consumption	500 and 2000 mg/kg/day in females: transient decreases in food consumption (17-22%) and body-weight gain (1-2 g loss vs. 7 g weight gain in controls). 2000 mg/kg/day in females: significant body weight loss on gestation day 15 (7.1%). All doses in males: transient decreases in food consumption, body weight, and body-weight gain. The reduced food consumption was attributed to the presumed decreased palatability of the diet due to the drug, although maternal/paternal toxicity of the test article cannot be ruled out.
Mating and fertility indices	No effects on male and female mating and fertility indices.
Necropsy findings/ Cesarean section data	500 and 2000 mg/kg/day in females: reduced number of corpora lutea/dam (9-11%), reduced number of implantation sites (13.2*-14.2 vs. 15.4 in controls), reduction in viable fetuses per dam (92.3-93.5%* vs. 96.6% in controls), and an increase in total resorptions (6.6*-7.7% vs. 3.5% in controls). Although statistically significant changes (*) were mainly observed in the 500 mg/kg/day group, the changes in the 500 and 2000 mg/kg/day groups were considered drug-related.
NOAEL/NOEL	The NOAEL for maternal toxicity was 30 mg/kg/day. The small changes in the reproductive tract of pregnant females appear to be secondary to maternal toxicity (i.e., not directly related to drug exposure). Therefore, the NOEL for female reproductive toxicity was 2000 mg/kg/day. The NOAEL for paternal toxicity was 150 mg/kg/day, and the NOEL for male reproductive toxicity was 750 mg/kg/day.

Source: Table prepared by the nonclinical reviewer.
Abbreviations: NOAEL, no observable adverse effect level; NOEL, no effect level

13.1.3.3.1.2. Embryo-Fetal Development

Study #SA5043/A Diet Admix Study of The Effect of Maralixibat On Embryo-Fetal Development in Rat

Key Study Findings

- The NOAEL for maternal and developmental toxicity was 1000 mg/kg/day ($AUC_{0\text{-}24h}$ 2020 ng•h/mL on Day 17 of gestation).

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 56. Rat Oral Embryo-Fetal Developmental Study Methods

Parameter	Method Details
Dose and frequency of dosing:	0, 50, 250, 1000 mg/kg/day
Route of administration:	Dietary (ad libitum)
Formulation/vehicle:	Not applicable.
Species/strain:	Sprague-Dawley rats (Crl:CD SD, IGS, BR)
Number/sex/group:	25
Satellite groups:	None
Study design:	Four groups of 25 mated female rats were treated with 0, 50, 250, or 1000 mg/kg/day maralixibat from day 6 to day 17 of gestation. Surviving females were sacrificed on gestation day 20 and were necropsied. Based on the amount of diet consumed, the actual doses in the three treatment groups were 47, 245, and 953 mg/kg/day.
Deviation from study protocol affecting interpretation of results:	None

Source: Prepared by the nonclinical reviewer.

Table 57. Observations and Results

Parameter	Results
Mortality	None
Clinical signs	250 and 1000 mg/kg/day: increased hair loss and scabbing on the forelimb, abdomen, and thorax; increased dried red material around the head in the mandibular area and nose.
Body weight/ food consumption	All doses: transient decreases in food consumption (up to 26%), likely caused by a decrease in palatability of the diet due to presence of test article. No effect on body weight.
Cesarean section data	No effect
Necropsy findings, offspring	50 and 1000 mg/kg/day: incidence of soft-tissue malformations was 0, 0.3, and 0.5% in the 0, 50 and 1000 mg/kg/day groups, respectively. The incidence of visceral malformations in the historical control data were 0-0.3%; therefore, the malformations in the treatment groups do not appear drug-related.
NOAEL	The NOAEL for maternal and developmental toxicity was 1000 mg/kg/day.
TK	Blood samples were collected at 6, 12, 18, and 24 h on gestation days 7 and 17. The concentration of maralixibat was determined using a validated liquid chromatographic/tandem mass spectrometric assay. Maralixibat was detected on gestation days 7 and 17. C_{max} increased in a less than dose-proportional manner on gestation days 7 and 17. The increase in AUC_{0-24h} was less than dose-proportional on gestation day 7, and greater than dose-proportional on gestation day 17. Accumulation occurred after repeated dosing. TK data are summarized in Table 58.

Source: Prepared by the nonclinical reviewer.
Abbreviations: AUC_{0-24h}, area under the curve from time 0 to 24 h; C_{max}, maximum concentration, NOAEL, no observable adverse effect level; TK, toxicokinetics

Table 58. Toxicokinetic Parameters in a Rat Embryo-Fetal Development Study

Gestation Day	Dose (mg/kg)	T_{max} [a] (h)	C_{max} (ng/mL)	$AUC_{0-24\ h}$ (ng•h/mL)
7	50	24	6.42	107
	250	18	8.97	182
	1000	24	68.7	709
17	50	6	14.0	169 (154) [b]
	250	6	23.8	459
	1000	12	169	2020

[a] Corresponds to 0600 (6 h), 1200 (12 h), 1800 (18 h) and (next day) 2400 (24 h).
[b] Value with outlier excluded.

Source: Applicant's report (#SA5043).
Abbreviations: AUC_{0-24h}, area under the curve from time 0 to 24 h; C_{max}, maximum concentration; T_{max}, time to maximum concentration

SA5061/A Study of the Effects of Maralixibat Administered Orally on Embryo-Fetal Development in Rabbits

Key Study Findings

- Significant decreases in maternal body-weight gain and food consumption were observed at 250 mg/kg/day.
- Abortion and a small increase in soft-tissue variations, including retrocaval ureter, absence of gallbladder, and accessory spleen, occurred at 250 mg/kg/day. These findings were associated with maternal toxicity.
- The NOAEL for maternal toxicity was 100 mg/kg/day.
- The NOAEL for developmental toxicity was 250 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 59. Methods of Oral Embryo-Fetal Developmental Study in Rabbit

Parameter	Method Details
Dose and frequency of dosing:	0, 25, 100, 250 mg/kg/day Once daily
Route of administration:	Gavage
Formulation/vehicle:	0.5% Methylcellulose w/v, 0.1% polysorbate w/v in distilled water
Species/strain:	New Zealand White female rabbits
Number/sex/group:	25
Satellite groups:	Six rabbits/group for toxicokinetic evaluation
Study design:	Four groups of 25 mated female rabbits were treated orally with 0 (vehicle), 25, 100, or 250 mg/kg/day Maralixibat from Day 7 to Day 18 of gestation. Females were sacrificed on gestation day 29 and Cesarean sections were performed.
Deviation from study protocol affecting interpretation of results:	None

Source: Prepared by the nonclinical reviewer.

Table 60. Observations and Results

Parameter	Results
Mortality	100 mg/kg/day: One female died on gestation day 8 due to an intubation error
Clinical signs	25 and 250 mg/kg/day: Abortion occurred in one female at 25 mg/kg/day, and in two females at 250 mg/kg/day on Days 25 and 29. The abortions at 250 mg/kg/day were associated with reduced food consumption and body weight. 250 mg/kg/day: Increased hair loss and scabbing on the dorsal thoracic area, dried red material at base of tail, decreased defecation, small fecal boli, absence of feces, and orange discoloration of urine.
Body weight/food consumption	100 and 250 mg/kg/day: Decrease in body weight was observed, which was reversed after cessation of drug. All doses: Maternal body-weight loss and decreased food consumption with significant decreases at 100 (body weight loss only) and 250 mg/kg/day (body-weight change: +6, -8, -19, and -70 g in the 0, 50, 100, and 250 mg/kg/day groups, respectively). Food consumption values were 192, 174, 182, and 122 g/animal/day in the 0, 50, 100, and 250 mg/kg/ day groups, respectively.
Necropsy findings/ Cesarean section data	250 mg/kg/day: Reductions in numbers of corpora lutea (10 vs. 11.3 per female in controls), implantation sites (6.8 vs. 8 per female in controls), and viable fetuses per litter (5.8 vs. 7.5 in controls) were observed. Increases in postimplantation losses (16% vs. 8.1% per litter in controls) and early resorptions (15.6% vs. 6.5% per litter in controls) were also observed. None of the changes was statistically significant. The numbers of postimplantation losses, early resorptions, and viable fetuses were within the historical control data range of the testing facility (0.6-23.1%, 0.6-21.9%, and 76.9-99.4% per litter, respectively).

Parameter	Results
Necropsy findings, offspring	All doses: An increase in soft-tissue variations was observed (12.5, 14.6, 22, and 18.8% at 0, 25, 100, and 250 mg/kg/day, respectively). Soft-tissue malformations included retrocaval ureter (0.4, 2.3, 2.4, and 3.4 at 0, 25, 100, and 250 mg/kg/day, respectively), absence of gallbladder (0.4, 0.8, 2.0, and 2.9 at 0, 25, 100, and 250 mg/kg/day, respectively), and accessory spleen (6, 5.6, 10, and 12.6 at 0, 25, 100, and 250 mg/kg/day, respectively). None of the changes was statistically significant. The incidence of retrocaval ureter, absence of gallbladder, and accessory spleen were within the range of historical control data (0-5.4%, 0-7.8%, and 6.2-24.6% per litter, respectively). It is likely that the small increase in soft-tissue variations at 250 mg/kg/day was due to maternal toxicity from the drug.
NOAEL	The NOAEL for maternal toxicity was 25 mg/kg/day based on reduced food consumption and body weight loss at ≥100 mg/kg/day. The NOAEL for developmental toxicity was 250 mg/kg/day.
TK	Blood samples were collected on days 7 and 18 of gestation to determine plasma levels of maralixibat using a validated liquid chromatographic/tandem mass spectrometric assay. Maralixibat was detected in plasma on days 7 and 18 of gestation in the 250 mg/kg dose group. The exposure increased with dose on gestation day 18. Accumulation occurred following repeated doses. Bioavailability for all doses was <1%. TK data are summarized in Table 61.

Source: Prepared by the nonclinical reviewer.
Abbreviations: NOAEL, no observable adverse effect level; TK, toxicokinetic

Table 61. Toxicokinetic Parameters in a Rabbit Embryo-Fetal Development Study

Gestation Day	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)
7	25	2.00	1.89	1.89
	100	NA[a]	NA[a]	NA[a]
	250	2.00	4.02	21.1
18	25	1.00	2.32	3.49
	100	1.00	6.19	51.5
	250	2.00	62.7	766
[a] All concentrations at this time point were below the lower limit of quantitation of 1.00 ng/mL.				

Source: Applicant's report #SA5061/A.
Abbreviations: AUC_{0-24h}, area under the curve from time 0 to 24 h; C_{max}, maximum concentration; T_{max}, time to maximum concentration

13.2. Individual Reviews of Studies Submitted to the NDA

13.2.1. Pharmacology

13.2.1.1. Primary Pharmacology

SD-5613 [Maralixibat] Timing and Dosing (Study # (b) (4) 99D1978)

Table 62. Methods and Results, (b) (4) 99D1978

Methods	Results
Male beagle dogs were divided into five groups and received the following oral maralixibat treatments by capsule: Group A:1 mg/kg (full meal fed after dosing) Group B: 1 mg/kg (half meal fed after dosing and half meal fed ~8 h later) Group C: 1 mg/kg (half meal fed before dosing and half meal fed after dosing); QD × 4 weeks Group D: 0.5 mg/kg (half meal fed following each dose); BID × 4 weeks Group E: vehicle (empty gelatin capsule) Three consecutive 24 h fecal samples were collected during the last 72 h period of the third week of treatment between 8:00 AM and 9:00 AM each day, prior to dosing and feeding.	Following 3 weeks of treatment, fBA excretion was increased by 440%, 405%, 510%, and 540% in groups A, B, C and D, respectively, compared to the vehicle group (E) (significant in all groups). Following 4 weeks of treatment, serum total cholesterol values were reduced by 25%, 13±2%, 24%, 20%, and 2% compared to pretreatment values in groups A, B, C and D and E, respectively (significant in groups A-D). Maralixibat was equally efficacious in increasing fBA and reducing serum total cholesterol independently of the mealtime and dosing frequency.

Source: Prepared by the nonclinical reviewer.
Abbreviations: BID, twice daily; fBA, fecal bile acids; QD, once per day

Maralixibat: Duration of Action and Time to Onset of Activity of a Single Oral Dose on Postprandial Total sBA in Beagle Dogs (Study # (b) (4) 99D1979)

Table 63. Methods and Results, Study (b) (4) 99D1979

Methods	Results
Experiments to measure time to onset of activity of maralixibat: • Maralixibat (capsule) was administered at 0, 0.01, 0.05, 0.2, and 1 mg/kg PO to dogs (n=6) at 1 h after feeding with a standard experimental meal. • Blood samples for sBA measurement were taken at -30, 0, 30, 60, 65, 70, 80, 90, 120, and 180 min from the time of feeding. Each dog served as its own control, and mean sBA levels were compared to the mean sBA level at 60 min (time when drug was administered). Experiments to measure the duration of action of maralixibat: • Method 1: Two Meals for Extended sBA Elevation: Maralixibat was administered at 0.05 and 0.2 mg/kg PO to 6 dogs at 1 h (when the sBA level had reached a maximum) after feeding with a meal. At 4 h after the meal was offered, a second meal half the size of the first was offered. – Blood samples for sBA measurement were taken at 0, 1, 1.5, 2, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 h from the time of offering the first meal. Mean sBA levels were compared to the mean sBA level at 1 h, with each dog serving as its own control. – The end of PD activity was considered to occur at the time point when the mean sBA value was not significantly lower than the 1 h mean value. • Method 2: One Meal and Extended Interval Between Dosing and Feeding – Six dogs were dosed with water or maralixibat at 0.05 mg/kg PO at 1.5 h prior to being fed, or with 0.05 or 0.2 mg/kg at 2 h prior to feeding. – Blood samples for sBA measurement were taken immediately before dosing (0 or 0.5 h), at feeding (2 h), 2.5, 3, 4, and 5 h after feeding. – Mean sBA levels were compared to the corresponding mean sBA levels in water-treated controls. – The end of PD activity was considered to occur at the first time point when the mean sBA value was not significantly lower than the corresponding control mean value.	Dose-dependent decreases in sBA levels were observed at 120 and 180 min following oral administration of maralixibat. sBA levels were significantly lower at 90, 120, and 180 min from the time of feeding at doses of 0.05, 0.2, and 1 mg/kg, respectively, compared to 60 min. In the first study design (method 1), maralixibat at 0.05 mg/kg significantly reduced sBA levels (versus peak value) at 4 h after feeding, after which sBA began to recover to normal levels. Maralixibat at 0.2 mg/kg significantly reduced sBA levels by 2 h after feeding, and this was sustained for an additional 6 h, at which time the experiment ended. In the second study design (method 2), maralixibat at 0.05 mg/kg significantly reduced sBA levels only at 1 h after feeding. Compared to the vehicle group, maralixibat at 0.2 mg/kg significantly reduced sBA at 1, 2, and 3 h after feeding, indicating that the higher dose had a longer duration of action than the 0.05 mg/kg dose. Thus, the PD activity of maralixibat on sBA appears to have a quick onset of action, and the duration of action is proportional to the size and timing of the dose administered with respect to the feeding time.

Source: Prepared by the nonclinical reviewer.
Abbreviations: PD, pharmacodynamic; PO, by mouth; sBA, serum bile acids

Evaluation of Maralixibat Efficacy in a Rat Model of Cholestasis (Study # (b) (4) 2013RES01)

Table 64. Methods and Results, Study (b) (4) 2013RES01

Methods	Results
Experimental cholestasis was induced in male SD rats by partial bile duct ligation. Six hours after surgery, rats (n=5-8/group) were treated with an oral solution (gavage) of maralixibat at doses of 0 (sterile water), 0.3, or 10 mg/kg/day for 14 days. A group of animals was subjected to a sham operation as the control group.	Three days following surgery, pBDL produced significant increases in sBA (23-fold), AST (4.2-fold), ALT (5-fold), ALP (8.8-fold), GGT (6.8-fold), and total bilirubin (68-fold), and a decrease in total fBA (3.5-fold). These findings were evidence of cholestatic liver injury. sBA and liver injury markers remained elevated in the vehicle control group during the 14-day treatment period.
Blood samples were collected on Days 3, 7, and 14 after surgery, for analysis of bile acids and biomarkers of liver injury. Fecal samples were collected for 24 h on Day 10 after surgery for analysis of total fBA.	Maralixibat produced decreases in sBA (44-96.7%), AST (32.3-69.5%), ALT (32.1-28.7%), ALP (14.4-80.5%), GGT (26.7-91.9%), and total bilirubin (20.6-98.4%), compared to the pBDL group dosed with the vehicle (data for Days 3 and 14). In general, the effects were dose- and duration-dependent and were significant. Maralixibat produced a 6.1-6.2 fold increase in fBA excretion at both doses without dose-dependency, compared to the pBDL vehicle-treated group. The values of most parameters, which decreased from Day 7 to Day 14, remained elevated compared to the sham group at the end of the study.

Source: Prepared by the nonclinical reviewer.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; fBA, fecal bile acids; GGT, gamma-glutamyl transferase; pBDL, partial bile-duct ligation; sBA, serum bile acids

Evaluation of SHP625 [Maralixibat] Efficacy in a Rat Model of Cholestasis: Comparison to, and in Combination with Ursodeoxycholic Acid (Study #R7311M-SHP625)

Table 65. Methods and Results, Study R7311M-SHP625

Methods	Results
Experimental cholestasis was induced in male SD rats by partial bile-duct ligation. Six hours after surgery, rats (n=8-9/group) were administered 1 mg/kg SHP625, 1 mg/kg UDCA, or 1 mg/kg SHP625 + 1 mg/kg UDCA by gavage for 14 days. The vehicles were sterile water for SHP625 and 0.8% carboxymethylcellulose + 5% propylene glycol for UDCA. The control group was treated with 0.8% carboxymethylcellulose + 5% propylene glycol. Blood samples were collected on Days 3, 7, and 14 after treatment for analysis of bile acids and biomarkers of liver injury. Fecal samples were collected for 24 h on Day 10 after treatment for analysis of total fBA.	After 3 days of treatment, SHP625 alone or in combination with UDCA did not produce significant decreases in sBA or biomarkers of liver injury. However, UDCA produced a significant decrease in ALP (28%). After 7 days of treatment, the combination of SHP625 and UDCA produced significant decreases in sBA (50%), total bilirubin (56%), and GGT (46%). After 7 days of treatment, UDCA alone produced significant decreases in sBA (35%) and total bilirubin (52%). After 14 days of treatment, SHP625 alone produced significant decreases in sBA (87%), ALP (26%), AST (62%), ALT (27%), GGT (90%), and total bilirubin (90%). After 14 days of treatment, UDCA alone produced significant decreases in sBA (82%), AST (54%), ALT (24%), and GGT (80%). After 14 days of treatment, the combination of SHP625 and UDCA produced significant decreases in sBA (91%), ALP (38%), AST (70%), ALT (36%), GGT (95%), and total bilirubin (90%). The magnitude of the decreases in ALP, ASP, ALT, and sBA was slightly greater in the combination group compared to the SHP625 or UDCA alone group. After 10 days of treatment, SHP625 or UDCA alone produced a 3.1- or 1.2-fold increase in fBA excretion, respectively. The combination of SHP625 and UDCA produced 3.7-fold increase in fBA excretion.

Source: Prepared by the nonclinical reviewer.
SHP625: code name for maralixibat
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; fBA, fecal bile acids; GGT, gamma-glutamyl transferase; sBA, serum bile acids; UDCA, ursodeoxycholic acid

Assessment of SHP625 [Maralixibat], SHP626 and SC-435 as Inhibitors of Human ASBT-Mediated Transport (Study #V7445M-SHP625)

The review below is limited to the effect of SHP625 on human ASBT-mediated transport since the other tested compounds are not relevant to this NDA submission.

Table 66. Methods and Results, Study V7445M-SHP625

Methods	Results
Madin-Darby canine kidney-II cells expressing human ASBT were treated with SHP625 at 1-300nM. The transporter substrate ([^{3}H]-taurocholate) was measured by radiometric assay.	The maximum inhibition of ASBT activity by SHP625 was 99.5% (at 100nM). The 50% inhibitory concentration was determined to be 7.04±0.756nM.

Source: Prepared by the nonclinical reviewer.
SHP625: code name for maralixibat
Abbreviation: ASBT, apical sodium-dependent bile acid transporter

13.2.1.2. Safety Pharmacology

Effect of Maralixibat on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study #MRXNC-003)

Table 67. Methods and Results, Study MRXNC-003

Methods	Results
Human embryonic kidney (HEK-293) cells expressing hERG potassium channels (n=3) were treated with maralixibat at 0.3, 1, 3, and 10 µmol/L. Vehicle was HEPES-buffered physiological saline with 0.3% DMSO. Terfenadine (60nM) was used as the positive control.	Maralixibat inhibited hERG current by -0.7±1.9% at 0.3 µmol/L and -1.2±0.9% at 1 µmol/L, as compared to -0.2±0.9% (n=3) in the vehicle control. The maralixibat values were not statistically significant compared to the vehicle control value. No usable data were obtained at the 3 and 10 µmol concentrations due to disruption of the giga-ohm seal required for successful patch clamp recordings (n=3 and n=6, respectively). The IC_{50} for the inhibitory effect of maralixibat on hERG potassium current was not calculated since no inhibition was observed (the negative inhibition values indicate increases in the hERG current).

Source: Prepared by the nonclinical reviewer.
Abbreviations: DMSO, dimethyl sulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hERG, human ether-à-go-go-related gene

13.2.2. Absorption, Distribution, Metabolism, Excretion/Pharmacokinetics

Absorption

The pharmacokinetic (PK) studies involving a single oral dose of maralixibat are summarized in Table 68, whereas repeated dose PK data (toxicokinetics) are summarized in the section on repeated dose toxicity studies (Sections 13.1.3.1 and 13.2.3.1.1). In general, oral absorption of the drug is very poor across species (e.g., mouse, rat, dog, guinea pig, and monkey). Bioavailability was less than 1% in adult rats and dogs following single and repeated oral dosing and was not affected by the presence of food. However, bioavailability was approximately 17%

in neonatal rat pups (postnatal day [PND] 7) and was <1% by PND 21. Systemic exposure (C_{max} and AUC) was low following oral administration, but increased with increasing dose after repeated dosing in mature animals. Absorption studies were conducted in portal vein-cannulated rats (Study #M3098259 and #M3098257). These studies demonstrated a first-pass-effect (FPE) of 7.88%, suggesting that the low bioavailability was not due to a strong FPE in this species. However, absorption studies conducted in portal vein-cannulated mongrel dogs (Study #M3098258 and #M3098260) showed that the FPE was 79.2%, suggesting that maralixibat is subject to a significant FPE.

Study reports are listed in Table 68 and the studies are described in Table 69, Table 70, Table 71, and Table 72.

Table 68. Pharmacokinetic Studies Reviewed

Study Title	Species	Study Number
Acute oral toxicity study of maralixibat in rat	Rat	SA4973
Determination of dose proportionality and the effect of food on systemic exposure after administration of maralixibat to rat		M3098255
Determination of absorption of maralixibat after oral administration in rat		M3098259
Determination of absorption of [^{3}H]maralixibat after oral administration in portal vein cannulated rat		M3098257
Mass balance of [^{3}H] maralixibat after oral administration in rat		M2098133
Absorption, metabolism, and excretion of [^{14}C]maralixibat following a 4-week dietary administration of maralixibat to rat		M2000269
Absorption, metabolism, and excretion of [^{14}C]maralixibat following a single intravenous or a single oral administration to rat		M2000112
Determination of dose proportionality and the effect of food and relative exposure of capsule and solution dosage forms after administration of maralixibat to beagle dog	Dog	M3098256
Acute oral toxicity study of maralixibat in dog		SA4948
Mass balance of [^{3}H]maralixibat after oral administration in beagle dog		M2098132
Absorption, metabolism, and excretion of [^{14}C]maralixibat following a single intravenous or a single oral administration to dog		M2000114

Source: Prepared by the nonclinical reviewer.

Table 69. Single-Dose Pharmacokinetics of Maralixibat (SD-5613) in Rat

Study Number	**SA4973**		**M3098255**			
Species/strain	Rat/ Sprague-Dawley					
Sex/number of animals	6M	6F	6M, 6F	6M, 6F	6M, 6F	6M, 6F
Feeding condition	Fasted		Fasted	Fasted	Fed	Fasted
Vehicle/formulation	Distilled water		0.2% Tween 80/Milli-Q water solution			
Method of administration	Oral gavage		Oral gavage			
Dose (mg/kg) (free-base equivalent)	M:1000	F: 2000	1	5	5	30
Sample	Plasma		Plasma			
Analyte	Maralixibat		Maralixibat			
Assay	LC/MS/MS		LC/MS/MS			
PK parameters						
Sex	M	F	M/F	M/F	M/F	M/F
T_{max} (h)	1	2	N/A	1	0.25	0.5
C_{max} (ng/mL)	153	143	<LOQ	2.33	3.40	5.15
AUC (ng•h/mL)	837	1170	<LOQ	6.09	12	3.95
Time interval for AUC (h)	0-24	0-24	N/A	0-8	0-8	0-8
Bioavailability (%)	0.0612	0.0428	N/A	0.129	0.254	0.014

Source: Prepared by the nonclinical reviewer.
LOQ, 2.38 ng free base/mL
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; F, female; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LOQ, limit of quantitation; M, male; N/A, not applicable; PK, pharmacokinetics; T_{max}, time to maximum concentration

Table 70. Single-Dose Pharmacokinetics of Maralixibat (SD-5613) in Rat (Continued)

Study Number	M3098259	M3098257	M2098133	M2000269	M2000112	
Species/strain	Rat/ (Sprague-Dawley)					
Sex/number of animals	6M, 6F	4M, 4F	9M, 9F	3M, 3F	6M, 6F	
Feeding condition	Fasted	Fasted	Fasted	Fasted	Fasted	
Vehicle/formulation	0.2% Tween 80/Milli-Q water solution	0.2% Tween 80/Milli-Q water solution	0.2% Tween 80/purified water solution	5% ethanol in sterile water suspension	5% ethanol in sterile water suspension	
Method of administration	Oral gavage	Oral solution delivered directly into duodenum	Oral gavage	Oral gavage	Oral gavage	
Dose (mg/kg) (free-base equivalent)	5	5	5	250	1000	2000
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	
Analyte	Maralixibat	[^{3}H] maralixibat	[^{3}H] maralixibat	[^{14}C] maralixibat	[^{14}C]maralixibat	
Assay	LC/MS/MS	LSC	LSC	LSC	LSC	
PK Parameters						
T_{max} (h)	2 (peripheral) 2 (portal)	1 (peripheral) 1 (portal)	0.25 (M) 0.5 (F) 1.0 (total)	1*	1 (M) 2 (F)	1 (F)
C_{max} (ng/mL)	9.25 (peripheral) 18.2 (portal)	25.2 (peripheral) 36.5 (portal)	14.1 (M) 10.7 (F) 8.46 (total)	91.5 (M) 136 (F)	879 (M) 237 (F)	<LOQ (M) 1180 (F)
AUC (ng•h/mL)	35.3 (peripheral) 61.8 (portal)	57.7 (peripheral) 80.0 (portal)	54.0 (M), 44.5 (F), 49.2 (total)	N/A	N/A	N/A
Time interval for AUC (h)	0-6	0-6	0-8	N/A	N/A	N/A
Bioavailability (%)	0.76	N/A	N/A	N/A	N/A	N/A

Source: Prepared by the nonclinical reviewer.
* Blood samples only collected at 1 and 8 h and PK parameters not calculated due to limited sampling.
LOQ, 0.44 mcg equiv/g
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; F, female; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LOQ, limit of quantitation; M, male; N/A, not applicable; PK, pharmacokinetics; T_{max}, time to maximum concentration; peripheral, peripheral blood plasma; portal, portal blood plasma

Table 71. Single-Dose Pharmacokinetics of Maralixibat (SD-5613) in Dog

Study Number	M3098256	SA4948					
Species/strain	Dog/Beagle						
Sex/number of animals	3F	2M; 2F	2M; 2F	2M; 2F	2M; 2F	2M; 2F	2M; 2F
Feeding condition	Fasted	Fasted					
Vehicle/formulation	Gelatin capsule	Gelatin capsule					
Method of administration	Oral	Oral					
Dose (mg/kg) (free-base equivalent)	7.5	50	200	400	600	800	1000
Sample	Plasma	Plasma					
Analyte	Maralixibat	Maralixibat					
Assay	LC/MS/MS	LC/MS/MS					
PK parameters							
Sex	F	M/F					
T_{max} (h)	5	2	2	2.75	2.25	2.5	1.5
C_{max} (ng/mL)	4.03	56.8	130	170	237	206	205
AUC (ng•h/mL)	32	132	244	690	908	735	637
Time interval for AUC (h)	0-24	0-24	0-24	0-24	0-24	0-24	0-24
Bioavailability (%)	0.059	0.0833	0.0385	0.0544	0.0477	0.029	0.0201

Source: Prepared by the nonclinical reviewer.
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; F, female; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LOQ, limit of quantitation; M, male; N/A, not applicable; PK, pharmacokinetics; T_{max}, time to maximum concentration

Table 72. Single-Dose Pharmacokinetics of Maralixibat (SD-5613) in Dog (Continued)

Study Number	M2098132	M2000114
Species/strain	Dog/Beagle	
Sex/number of animals	3F	3M, 3F
Feeding condition	Fasted	Fasted
Vehicle/formulation	0.2% Tween 80/purified water solution	5% ethanol in sterile water solution
Method of administration	Oral gavage	
Dose (mg/kg) (free-base equivalent)	7.5	50
Sample	Plasma	Plasma
Analyte	[^{3}H]maralixibat	[^{14}C]maralixibat
Assay	LSC	LSC
PK parameters		
T_{max} (h)	0.920	1 (M) 1 (F)
C_{max} (ng/mL)	33.8	66.3 (M), 70.3 (F)
AUC (ng•h/mL)	151/336	N/A
Time interval for AUC (h)	0-8/0-24	N/A
Bioavailability (%)	N/A	N/A

Source: Prepared by the nonclinical reviewer.
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; F, female; LSC, liquid scintillation counting; M, male; N/A, not applicable; PK, pharmacokinetics; T_{max}, time to maximum concentration

Distribution

Tissue Distribution Studies

Studies that evaluated tissue distribution in mouse (oral or intravenous [IV] administration), rat (IV administration), dog (IV administration), monkey (IV administration), and rabbit (oral

administration) were not reviewed because the IV route is not the intended route in humans, and mouse, rabbit, and monkey were not used in the pivotal toxicity studies.

Study Title: Mass Balance of [^{3}H]Maralixibat after Oral Administration in Rat (Study #M2098133)

Sprague-Dawley rats (n=3/sex) were administered a single dose of 5 mg/kg [^{3}H]maralixibat by oral gavage. Blood samples were collected predose and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 h after dosing. Urine and feces samples were collected predose (approximately -24 to 0 h) and at 24 h intervals to 168 h postdose. GI tract tissues were collected at 168 h after administration. LSC was used to quantify radiolabeled maralixibat in samples.

The plasma concentrations of total radioactivity were low at all time points, declined rapidly, and were below the limit of detection (3.80 ng equivalents/mL or eq/mL) by 12 h postdose. The mean peak concentration of radioactivity in plasma was 8.46 ng free base eq/mL observed at 1 h postdose. The mean area under the concentration-time curve from time 0 to 8 h was 49.2 ng eq•h/mL. The concentrations in the cellular fractions were below the limit of detection (11.3 ng eq/g) at all time points.

Low concentrations were detected in all tissues, with the highest in the ileum in male and female rats. The mean total percentage of administered radioactivity remaining in tissues at 168 h postdose was 0.01% or less in all animals. The tissue distribution data are listed in Table 73.

Table 73. Distribution of [^{3}H]Maralixibat at 168 h in Male and Female Rats After Oral Administration

Tissue / Sex	Concentration (ng equiv/g)				
	Stomach	Duodenum	Jejunum	Ileum	Colon
Male	10.8	3.62	9.44	81.6	15.1
Female	5.86	< LOD	< LOD	9.54	< LOD

Source: Applicant's report (#M2098133).
Abbreviation: LOD, limit of detection (<1.36 ng eq/g).

Following administration, the total percentage of the radioactive dose recovered in urine, feces, cage wash, and cage wipes was 92.5%, with 64.4% excreted in feces and 10.4% in urine. The relatively high percentage of radioactivity detected in urine may be due to fecal contamination of urine samples, since 17.6% of the administered dose was found in cage wash and cage wipe. An average of 54% of the dose was eliminated within the first 24 h postdose.

Study Title: Tissue Distribution and Mass Balance of [^{14}C]Maralixibat Following a Single Oral Dose to Rat (Study #M2098359)

Long Evans male rats were administered a single dose of 5 mg/kg [^{14}C]maralixibat by oral gavage. Blood and tissue samples (e.g., gastrointestinal [GI] tract, brain, heart, eye, liver, kidney, adrenal gland, lung, spleen, testes, bone, skin, fat, and muscle) were collected predose and at 0.25, 0.5, 1, 4, 8, 12, 24, 48, 72, 96, and 168 h post-dose. Urine, feces, and expired air were collected at specified intervals from the animals sacrificed at 168 h postdose. Samples were analyzed by LSC. The lower limit of quantitation (LLOQ), based on control tissue weights, was determined using the background values for that analysis batch. Thus, the LLOQ values were different among the tissue types analyzed.

The maximum mean concentrations of radioactivity in blood and plasma were 1.75 and 4.71 ng free-base equivalents/g, respectively, and occurred at 4 h postdose. The concentrations in blood and plasma were near or below the LLOQ to 4 h postdose and were not detectable thereafter. Radioactivity was not detected in the cellular fraction of blood.

The highest concentration of radioactivity was observed in tissues of the GI tract. In the non-GI tissues, the highest concentrations were observed in pancreas (633 ng eq [^{14}C]maralixibat/g; 0.5 h), liver (447 ng eq/g; 0.5 h), and adrenal glands (260 ng eq/g; 0.5 h). By 12 h after dosing, radioactivity was detected in 17 of 34 tissues collected. Tissue to plasma concentration ratios were calculated for all collected tissues in which radioactivity was detectable up to 4 h postdose. The mean tissue to plasma concentration ratios were greater than one in most of the tissues from 0.5 to 1 h postdose, and less than one in the majority of the tissues (21 of 33 tissues) at 4 h postdose.

The GI tissue distribution data and GI tissue to plasma ratios are shown in Table 74.

Table 74. Distribution and Tissue: Plasma Ratio of [^{14}C]Maralixibat in Rats in Gastrointestinal Tissues After Oral Administration

Time (hour) / Tissues	Concentration (ng equiv/g)									
	0.5	1	4	8	12	24	48	72	96	168
Small intestine	19,600	14,500	20,400	318	212	110	34.1	13.0	10.3	11.1
Stomach	14,500	24,400	6,250	194	202	66.1	10.0	6.73	5.49	2.55
Cecum	79.2	74.9	2,740	13,800	852	79.1	10.5	5.56	1.73	2.33
Colon	134	24.9	407	4,420	594	79.1	9.60	7.19	7.90	3.89
	[Tissue:plasma ratio]									
Small intestine	5,070	6,120	6,160	-	-	-	-	-	-	-
Stomach	3,910	9,990	1,510	-	-	-	-	-	-	-
Cecum	22.9	25.5	715	-	-	-	-	-	-	-
Colon	42.2	12.6	102	-	-	-	-	-	-	-

Source: Applicant's report #M2098359.

The area under the concentration-time curve from time 0 to 4 h values for blood and plasma were 4.82 and 13.4 h•ng eq/g, respectively. The tissues with the lowest area under the concentration-time curve from time 0 to infinity values, excluding blood and plasma, were brain and testes (14.6 and 23.6 h•ng eq/g, respectively). The highest area under the concentration-time curve from time 0 to infinity values were observed in the small intestine, stomach, cecum, and colon (91,600, 64,100, 61,300, and 23,200 h•ng eq/g, respectively).

Overall, almost all of the administered radioactivity was associated with the GI tract tissues and contents, with only minimal radioactivity associated with non-GI tissues. From 0.5 to 4 h, 89.7% to 90.0% of the dose was associated with the GI tissues and contents whereas only 0.72 to 0.16% was associated with all non-GI tissues. By 72 h, 0.92% was associated with the GI tract and contents and <0.005% was associated with all non-GI tissues. Radioactivity was not detectable in most of the non-GI tract tissues at 168 h postdose.

At 168 h postdose, 88.3% and 5.88% of the administered radioactivity was detected in the feces and urine, respectively. Radioactivity was not detected as carbon dioxide or in expired organic volatiles. [^{14}C]Maralixibat-derived radioactivity was poorly absorbed and was excreted primarily in feces; therefore, a fraction of the radioactivity in urine was probably due to fecal

contamination. Most of the radioactivity was excreted at 48 h, with mean values of 81.1% in feces and 2.44% in urine.

Study Title: Mass Balance of [^{3}H]Maralixibat after Oral Administration in Beagle Dog (Study #M2098132)

Three female dogs received a single dose of [^{3}H]maralixibat (7.5 mg/kg free-base equivalent) by oral gavage. Blood samples were collected predose and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 24, 48, 72, 96, and 120 h after dosing. Urine and feces samples were collected predose (approximately -24 to 0 h) and at 24 h intervals to 168 h postdose. At the time of sacrifice, the stomach, duodenum, jejunum, ileum, and colon were collected. Total radioactivity in samples was analyzed by LSC.

The concentrations of total radioactivity in plasma were low at all time points and declined rapidly following the oral dose of [^{3}H]maralixibat. The mean C_{max} and T_{max} were 33.8 ng eq/mL and 0.92 h, respectively. The mean area under the concentration-time curve from time 0 to 8 h and $AUC_{0\text{-}24h}$ were 151 and 336 ng eq•h/mL, respectively.

Concentrations of total radioactivity in blood cellular fractions were low at all time points and were below the limit of detection (8.40 ng eq/g) by 1.5 h postdose. The mean C_{max} in the cellular fraction was 4.37 ng eq/g at 0.5 h postdose. Radioactivity concentrations in the cellular fraction were lower than the corresponding concentrations in plasma at all time points.

The percentage of total radioactivity in collected tissues was <0.005%. The mean concentrations of radioactivity in the stomach, ileum, and jejunum were 21.4, 3.79, and 1.38 ng eq/g, respectively. The duodenum and colon radioactivity levels were below the limit of detection (4.20 ng eq/g).

The mean total percentage of radioactive dose recovered in urine, feces, cage wash, and cage wipes was 95.3%, with 94.3% excreted in feces and 0.1% in urine. An average of 80.6% of the dose was eliminated in feces within the first 24 h postdose. Thus, fecal excretion was the primary elimination route.

Study Title: Protein Binding of Maralixibat to Mouse, Rat, Guinea Pig, Rabbit, Cynomolgus Monkey, and Dog Plasma and Human Serum Albumin and Alpha-1-Acid Glycoprotein (Study #M3099225)

Plasma was obtained from mouse, rat, guinea pig, rabbit, cynomolgus monkey, dog, and human. Human serum albumin solution and alpha-1-acid glycoprotein solution were purchased from [redacted] (b) (4) The binding of [^{14}C]maralixibat to total human plasma proteins and human albumin and alpha-1-acid glycoprotein at 0.025, 0.25, 2.5, and 25 µg/mL was determined using an ultracentrifugation method. The concentrations of [^{14}C]maralixibat in plasma and protein fortified buffer were determined by LSC.

The plasma protein binding of maralixibat in mouse, rat, guinea pig, rabbit, dog, cynomolgus monkey, and human was >80%. The binding of maralixibat to human serum albumin and alpha 1-acid glycoprotein was >91% and >93%, respectively. The binding to plasma proteins and specific proteins was relatively consistent and concentration independent over the concentration range of 0.025 to 25 µg/mL maralixibat. The percentage of protein-bound [^{14}C]maralixibat in plasma and protein fortified buffer solutions is summarized in Table 75.

Table 75. Mean Percentage of Protein-Bound [^{14}C]Maralixibat in Plasma and Protein-Fortified Buffer Solutions

[^{14}C]SD-5613 (µg/mL)			
Matrix	0.250	2.50	25.0
Human Serum Albumin	97.8	98.5	98.1
Alpha 1-Acid Glycoprotein	98.5	99.3	96.2
Mouse	95.9	97.3	96.0
Rat	90.4	89.6	86.8
Guinea Pig	88.7	90.8	91.6
Rabbit	84.2	90.6	91.6
Cynomolgus Monkey	90.3	92.3	92.5
Dog	92.4	91.3	91.5
Human	90.6	91.2	90.2

Source: Applicant's report #M3099225.
Abbreviations: SD-5613, maralix bat

Study Title: Erythrocyte Partitioning of SD-5613 in Rat, Dog, and Human Blood (Study #M3099280)

Whole blood was obtained from rat, dogs and humans The erythrocyte partitioning of [^{14}C]maralixibat was studied in rat, dog, and human blood at 0.025, 0.25, 2.5, and 25 µg/mL using a digestion method. Concentrations of [^{14}C]maralixibat in plasma and whole blood were determined by LSC. The mean erythrocyte partitioning at 0.025 µg/mL [^{14}C]maralixibat in rat, dog, and human was 30.4, 37.9, and 41.4%, respectively, at 0 h. At 2 h, the mean erythrocyte partitioning at 0.025 or 25 µg/mL [^{14}C]Maralixibat in human was 44.5% and 36.9%, respectively. Decreased partitioning was observed as the concentration increased, possibly indicating that red blood cell partitioning is saturable.

13.2.3. General Toxicology

13.2.3.1. General Toxicology: Additional Studies

13.2.3.1.1. A 3-Month Study of SHP625 [Maralixibat] by Oral Gavage in Sprague-Dawley Rats (Study #R7834M-SHP625)

This study was used to support the qualification of impurities [redacted]$^{(b)\ (4)}$.

Table 76. Information, Study R7834M-SHP625

Study Features and Methods	Details
Conducting laboratory and location: GLP compliance: yes	(b) (4)
Methods	
Dose and frequency of dosing:	5, 30, 75, and 150 mg/kg/day in males 5, 30, 150, and 500 mg/kg/day in females Once daily
Route of administration:	Oral gavage
Formulation/vehicle:	Deionized water
Species/strain:	Rats/Sprague-Dawley
Number/sex/group:	10 in main study; 3-9 in toxicokinetic (TK) groups
Age:	7 weeks
Satellite groups/ unique design:	TK/none
Deviation from study protocol affecting interpretation of results:	No

Source: Prepared by the nonclinical reviewer.
Abbreviation: GLP, good laboratory practices

Table 77. Observations and Results, Study R7834M-SHP625

Parameters	Major Findings
Mortality	None
Clinical signs	None
Body weight	None
Ophthalmoscopy	None
Electrocardiogram	Not applicable
Hematology	Nonsignificant increase in prothrombin time in the 150 mg/kg/day group males (+4.8%).
Clinical chemistry	None
Urinalysis	None
Gross pathology	None
Organ weights	None
Histopathology Adequate battery: Yes	Increased incidence of chronic progressive nephropathy in males (7/10 M at 150 mg/kg/day vs. 3/10 M in controls). Increased incidence of minimal monocyte infiltration in liver in males (6/10 M at 150 mg/kg/day vs. 3/10 M in controls). Minimal to mild cystic degeneration in adrenal cortex in the 500 mg/kg/day female group (2/10 F vs. 0/10 F in controls). Minimal to mild muscle regeneration in esophagus in the 500 mg/kg/day female group (2/10 F vs. 0/10 F in controls). These microscopic findings were observed in the high-dose groups. However, their severity was low, and their incidence was similar or slightly higher than in the control group. Therefore, these changes are not considered to be adverse. The NOAEL was 150 mg/kg/day for males and 500 mg/kg/day for females.

Parameters	Major Findings
Toxicokinetics	Plasma drug levels were measured at 1, 2, 3, 5, 8, and 24 h postdose on Days 0 and 90. Toxicokinetic parameters are presented in Table 78. The exposure to maralixibat (C_{max} and AUC_{0-t}) in both sexes generally increased with dose level over the entire dose range, although the increase in exposure was less than dose-proportional. Sex-related exposure trends were inconsistent. Overall, there was no accumulation following repeated doses.

Source: Prepared by the nonclinical reviewer.
Abbreviations: AUC_{0-t}, area under the curve from time 0 to the last measurable concentration using the linear-log trapezoidal rule; C_{max}, maximum concentration; F, female; M, male; NOAEL, no observable adverse effect level

Table 78. Summary of Toxicokinetic Parameters

Dosage	AUC_{0-t} (ng•hr/mL)		C_{max} (ng/mL)		T_{max} (hr)	
	Day 0	Day 90	Day 0	Day 90	Day 0	Day 90
Males						
5 mg/kg	14.8	13.7	11.5	1.60	1.00	24.00
30 mg/kg	35.3	16.7	7.19	2.34	1.00	3.00
75 mg/kg	13.9	13.7	3.32	2.07	1.00	3.00
150 mg/kg	45.2	21.6	4.42	3.03	1.00	3.00
Females						
5 mg/kg	6.91	4.45	3.74	1.34	1.00	3.00
30 mg/kg	9.73	17.9	2.35	2.21	1.00	1.00
150 mg/kg	45.8	37.4	7.53	6.16	2.00	1.00
500 mg/kg	51.8	210	6.38	24.0	3.00	2.00

Source: Applicant's study report #R7834M-SHP625.
Abbreviations: AUC_{0-t}, area under the curve from time 0 to the last measurable concentration using the linear-log trapezoidal rule; C_{max}, maximum concentration; T_{max}, time to maximum concentration

13.2.3.2. Prenatal and Postnatal Development

13.2.3.2.1. An Oral (Dietary) Study of the Effects of SHP625 [Maralixibat] on Pre- and Postnatal Development, Including Maternal Function, in Rat

Key Findings

- Maralixibat at 250 and 750 mg/kg/day produced significant increases in body-weight gain during pregnancy, which was associated with increased food consumption.
- Maralixibat produced a dose-dependent and significant increase in body weight generally throughout lactation, and an increase in body-weight gain during lactation days (LDs)1 to 4. These effects were associated with increased food consumption in the F0 females.
- From PND 17 to 21, the pups (F1) had reduced body weight and body-weight gain. This was attributed to consumption of the maternal feed, which was the test article.
- Exposure to maralixibat increased with dietary concentrations in the F0 dams during the gestation and lactation periods.
- SHP625 was detected in plasma in pups on PND 4, indicating that SHP625 was excreted in milk.
- The NOAEL for pre- and post-natal development and maternal toxicity was 750 mg/kg/day (highest dose tested).

Study no.:	(b) (4) 771155/R7114M-SHP625
Study report location:	Module 4 Nonclinical study reports
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot number, and percentage purity:	M020719-CA15-0483 cryst 1#1; 99.53%
Enhanced PPND study:	N

Table 79. Study Information

Study Features and Methods	Details
Methods:	
Doses:	50, 250, 750 mg/kg/day The target dose levels of 50, 250, and 750 mg/kg/day corresponded to actual average doses of 56, 309, and 903 mg/kg/day, respectively, during gestation and 54, 269, and 762 mg/kg/day, respectively, during lactation, based on the amount of diet (feed) consumed.
Frequency of dosing:	N/A (dietary administration ad libitum was used)
Number/sex/group:	25
Dose volume:	N/A
Formulation/vehicle:	Dietary mixture
Route of administration:	ORAL
Species:	Rats
Strain:	Crl:CD(SD) rats
Comment on study design and conduct:	Mated female rats (25/group) were administered the test article in diet (ad libitum) from gestation day 6 to lactation day 21. All animals were allowed to deliver and rear their offspring to lactation day 21. The F1 animals were not intentionally exposed to the test article at any time during the study. Regardless, it appeared that F1 pups consumed the maternal treatment diet during the late part of the lactation period.
Dietary formulation analysis:	The dietary formulations were within the (b) (4) SOP range for dietary admix formulations (85% to 115%).

Source: Prepared by the nonclinical reviewer.

Observations and Results

F0 Dams

Mortality

One female in the 750 mg/kg/day group was found dead on GD 21. No significant clinical findings were observed prior to death. Seven dead fetuses were found in utero at necropsy. Additional macroscopic findings in this animal included red matting in the urogenital area, dark-red contents in the stomach, uterus, and vagina; and dark-red areas of the thymus. The death was not considered to be drug-related, although the cause of death could not be determined.

One female in the 250 mg/kg/day group was found dead on lactation day 2. No significant clinical findings were noted for this female. Findings from necropsy included cannibalized pups in the stomach and red fluid in the thoracic cavity.

Clinical Signs

Drug-related clinical signs included an increased incidence of red material around the nose at all doses.

Body Weight

There were no effects on body weight during the gestation period.

Maralixibat produced significant increases in body-weight gain during GD 9 to 12 (44% at 750 mg/kg/day) and GD 6 to 20 (9.7% at 250 mg/kg/day).

Maralixibat produced a significant, dose-dependent increase in body weight during lactation (5.7%, 6.5%, and 9.2% at 50, 250, and 750 mg/kg/day, respectively, on LD 21). A significant increase in body-weight gain was observed during LD 1 to 4 without a dose effect (87.5%, 87.5%, and 37.5% at 50, 250, and 750 mg/kg/day, respectively).

Food Consumption

Maralixibat produced significant increases in food consumption (g/animal/day) during GD 9 to 12 (24% at 750 mg/kg/day) and GD 6 to 20 (11.5% at 250 mg/kg/day).

Maralixibat at 50 and 250 mg/kg/day produced a significant increase in food consumption (g/animal/day) during LD 1 to 10 (13% and 11.1%, respectively).

F0 dams in the treatment groups had a large number of feed spills as a result of scratching the feed in the jars. It is likely that the feed spills inflated the measurements of food consumption in the treatment groups, primarily during the gestation period.

Gestation Duration and Parturition

There were no drug-related effects.

Necropsy

There were no drug-related macroscopic findings at LD 21.

F1 Generation

Preweaning

PND 0 Litter Data and Postnatal Survival

Maternal drug exposure at all doses had no drug-related effects on number of pups born, live litter size, percentage of males per litter at birth, or postnatal survival.

The number of pups (litters) that were found dead were 6 (6), 9 (7), 11 (7), and 10 (5) in the control, 50, 250, and 750 mg/kg/day groups, respectively. Several pups were missing and presumed to have been cannibalized (6, 3, 2, and 13 pups in the control, 50, 250, and 750 mg/kg/day groups, respectively). Among the pups that were found dead, absence of milk in

stomach was observed in 0, 4, 3, and 4 pups in the control, 50, 250, and 750 mg/kg/day groups, respectively.

F1 Body Weights

During PND 17 to 21, the pups had reduced body weight compared to controls. This change was significant in the 750 mg/kg/day maternal group (6.5% in males and 5.9% in females on PND 21). Reduced body weight was attributed to pups consuming the maternal diet, resulting in direct exposure to the test article. During the same period, pups had significant decreases in body-weight gain (13.8%, 19.4%, and 14.9% in males in the 50, 250 and 750 mg/kg/day groups, respectively; 9%, 18.9%, 17.4% in females in the 50, 250, and 750 mg/kg/day groups, respectively).

F1 Pup Necropsy on PND 21

Dilated renal pelvis was noted in 1, 2, 1, and 3 pups in the control, 50, 250, and 750 mg/kg/day groups, respectively. In addition, two pups in the 750 mg/kg/day group had distended ureters. These changes were not considered drug-related based on the low incidence.

Postweaning

Mortality and Clinical Signs

There were no clinical findings in the F1 generation (offspring) related to maternal doses of maralixibat.

Body Weight and Body-Weight Gain

Maternal doses had no effect on F1 body weight or body-weight gain during PND 21 to 84, PND 21 to 119, mating, gestation, and lactation (LD 1 to 4).

F1 Developmental Landmarks, Sensory Function, and Behavioral Testing

Sexual Maturation

Maternal treatment with maralixibat had no effects on balano-preputial separation or vaginal patency in the offspring.

Auditory Startle Response

An auditory startle response test was performed on 1 rat/sex/litter (from 25 litters/group) on PND 20 and 60. Maternal treatment with maralixibat had no effects on auditory startle response.

Motor Activity

Motor activity was assessed for 1 rat/sex/litter (from 25 litters/group) on PND 21 and 61. Maternal dosing with maralixibat had no effects on motor activity.

Biel Maze Swimming Studies

To evaluate learning and memory, a water-maze test was conducted using an 8-unit T-maze on PND 22 and 62 for 1 rat/sex/litter (from 25 litters/group). Maternal treatment with maralixibat had no effects on the performance of offspring.

Reproductive Performance

Maternal dosing with maralixibat had no effect on reproductive performance in the F1 generation (Table 80).

Table 80. Reproductive Performance

Parameter	Dosage Level (mg/kg/day)				CRL HC[a]
	0	50	250	750	Mean (Range)
Male Mating Index (%)	100.0	100.0	100.0	100.0	95.5 (84.0-100.0)
Female Mating Index (%)	100.0	100.0	100.0	100.0	98.1 (92.0-100.0)
Male Fertility Index (%)	96.0	100.0	100.0	95.8	90.4 (60.0-100.0)
Female Fertility Index (%)	96.0	100.0	100.0	95.8	93.1 (60.0-100.0)
Male Copulation Index (%)	96.0	100.0	100.0	95.8	94.2 (71.4-100.0)
Female Conception Index (%)	96.0	100.0	100.0	95.8	93.8 (65.2-100.0)
Estrous Cycle Length (days)	4.2	4.4	4.2	4.0	4.3 (4.0-5.0)
Pre-Coital Interval (days)	2.5	2.5	2.4	2.1	3.2 (2.3-4.8)

[a] Charles River historical control data
None significantly different from the control group.
Source: Applicant's report # (b) (4)-771155/R7114M-SHP625.

Gestation Length and Parturition

Maternal dosing with maralixibat had no effects on gestation length or parturition in the F1 generation.

Scheduled Necropsy

All surviving females with viable pups on LD 4 were subjected to gross examination (necropsy). Administration of maralixibat in the F0 females had no effects on the number of corpora lutea, former implantation sites, or unaccounted-for sites in the F1 generation.

Maternal doses did not produce drug-related macroscopic findings in the F1 generation, including offspring that were sacrificed prior to testing of reproductive performance and the offspring used in the reproductive performance test (sacrificed on LD 4).

F2 Generation

PND 0 Litter Data and Postnatal Survival

Maternal treatment with maralixibat (F0 females) had no effect on the number of F2 pups born, live litter size, percentage of males per litter at birth, or postnatal survival (PND 0, 0 to 1, and 1 to 4).

General Physical Condition

Maternal dosing with maralixibat (F0 females) had no effects on clinical findings.

Litter Weight and Weight Gain

Maternal treatment with maralixibat (F0 females) had no effect on litter weight or litter weight gain.

Necropsy of Unscheduled Death

The number of F2 pups (litters) found dead or euthanized in extremis was 12 (6), 7 (6), 27 (9), and 11 (9) in the control, 50, 250, and 750 mg/kg/day groups, respectively. No macroscopic findings were observed in these animals.

Toxicokinetics and Lactation (Exposures)

Blood samples were collected from five F0 females in each group on GD 17 and LD 4 at 2 h±15 min following the scheduled time for turning on lights in the study room. Blood samples were also collected from two culled pups/sex from five litters/group on PND 4 (i.e., litters from the F0 females used in the toxicokinetic [TK] analysis).

The plasma concentrations of maralixibat increased with dietary concentrations in the F0 dams during gestation and lactation, and in plasma from pups on PND 4. Maternal exposures were less than the offspring exposure on PND 4. The data support the conclusion that maralixibat is excreted in milk. The levels of maralixibat in dams and pups are listed in Table 81 and Table 82.

Table 81. Mean Plasma Maralixibat Concentration in Dams During Gestation and Lactation

	Dose Groups (mg/kg/day)		
Study Day	**50**	**250**	**750**
Gestation Day 17, ng/mL±SD	2.06±1.44	5.14±1.16	8.65±1.88
Lactation Day 4, ng/mL±SD	0.76±0.26	3.54±1.12	9.62±1.75

Source: Prepared by the nonclinical reviewer.
Abbreviation: SD, standard deviation

Table 82. Mean Plasma Maralixibat Concentration in Pups From Treated Dams on PND 4

	Maternal Dose Groups (mg/kg/day)		
Study Day (sex)	**50**	**250**	**750**
PND 4 (males), (ng/mL)±SD	5.05±4.25	12.4±3.37	65±40.2
PND 4 (females), (ng/mL)±SD	5.28±3.99	12.4±6.22	63.6±18.7
PND 4 (sexes combined), (ng/mL)±SD	5.17±3.89	12.4±4.72	64.3±30.6

Source: Prepared by the nonclinical reviewer.
Abbreviations: PND, postnatal day; SD, standard deviation

13.2.3.3. Juvenile Animal Toxicology

13.2.3.3.1. Study Title: An Oral (Gavage) Juvenile Toxicity Study of Maralixibat in Sprague-Dawley Rats, With a Recovery and a Toxicokinetic Phase

Key Findings

- Unscheduled deaths occurred in males at 250 mg/kg/day due to septicemia.
- Overall, significant increases in body weight and body-weight gain were observed mainly in the 100 and 250 mg/kg/day groups.
- All doses produced significant increases in red cell indices.

- Drug-related increases in ALP at 50, 100, and 250 mg/kg/day and in phosphorus at 100 and 250 mg/kg/day.
- Males at ≥100 mg/kg/day showed trends for increases in distal femur bone mineral content (BMC), associated with significant increases in bone mineral density (BMD) at 250 mg/kg/day.
- Females at 250 mg/kg/day had a significant increase in distal femur total slice area, associated with significant increases in BMC and BMD.
- A minimal increase in systemic exposure to maralixibat occurred with increasing dose increasing dose. The systemic exposure on Days 8 and 14 (PND 14 and 20, respectively) was substantially lower than the exposure on Day 1 (PND 7), which is typical for toxicity studies in neonatal/juvenile rats.
- The target organ of toxicity was bone. However, the bone effects occurred at extremely high AUC multiples (>500 based on the lowest rat AUC) of the human AUC; therefore, they are not clinically relevant.
- The NOAEL in both sexes was 100 mg/kg/day, based on septicemia, death, and bone effects in the 250 mg/kg/day males and bone effects in the 250 mg/kg/day females.

Study no.:	2939-001/MRX-NC-001
Study report location:	Module 4
Study initiation date:	April 30, 2020
Conducting laboratory and location:	(b) (4)
Duration:	14
Duration units:	days
GLP compliance:	Y
Drug, lot number, and percentage purity:	CA16-1118; 99.8%

Scientific Justification for Study

None.

JAS-Specific Toxicity

Bone

Table 83. Study Information

Methods	
Doses:	50, 100, 250 mg/kg/day
Frequency of dosing:	Once daily
Number/sex/group:	20
Dose volume:	5 mL/kg
Formulation/vehicle:	10, 20, 50 mg/mL/deionized water
Route of administration:	Oral gavage
Species:	Rat

Methods	
Strain:	CD1(ICR)
Age at start of experiment:	Postnatal day (PND) 7
Period of development studied:	PND 7-21 (dosing) PND 22-49 (recovery)
Comment on study design and conduct:	Rats were dosed for 15 days, followed by a 28-day recovery period (Table 84); 15-60/sex/group in toxicokinetic animals.
Dosing solution analysis:	All acceptance criteria were achieved.

Source: Prepared by the nonclinical reviewer.

Table 84. Study Design

Group No.	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Animals	
				Males	Females
Main Study					
1	0	0	5	20[a]	20[a]
2	50	10	5	20[a]	20[a]
3	100	20	5	20[a]	20[a]
4	250	50	5	20[a]	20[a]
Toxicokinetic					
5	0	0	5	15[b]	15[b]
6	50	10	5	60[b]	60[b]
7	100	20	5	60[b]	60[b]
8	250	50	5	60[b]	60[b]

[a] 10 animals/sex/group were maintained for a 28-day recovery period.
[b] 6 extra animals/sex/group were included to be used as replacements.

Source: Applicant's report #2939-001/MRX-NC-001.

Table 85. Dosing Schedule

Group No.	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Main Study Animals			
				Terminal		Recovery	
				Males	Females	Males	Females
1 Control	0	5	0	10	10	10	10
2 Low Dose	50	5	10	10	10	10	10
3 Mid Dose	100	5	20	10	10	10	10
4 High Dose	250	5	50	10	10	10	10

Source: Applicant's report #2939-001/MRX-NC-001.

Dose selection was based on a dose range-finding study (CR00771280/R9976M-SHP625) which demonstrated drug-related mortality and moribundity in females at 500 and 1000 mg/kg/day, associated with adverse clinical signs, reduced body weight, and reduced body-weight gain. Drug-related changes in clinical chemistry parameters included increased ALP and triglycerides in the 1000 mg/kg/day female group and decreased mean globulin in the 250, 500, and 1000 mg/kg/day female groups. Based on these results, 250 mg/kg/day was selected as the high dose, and the mid- and low-dose levels were selected to assess graded effects.

Observations and Results

Mortality

Observations of animals found dead or euthanized in moribund condition are listed in Table 86.

Table 86. Unscheduled Euthanasia and/or Deaths During the Course of the Study

Animal Number/Sex	Dose Level (mg/kg/day)	Fate Day	Fate	Clinical Observations	Macroscopic observations
6031/M	50 (TK)	12	FndDd	NA	All tissues normal
6526/F	50 (TK)	12	FndDd	NA	All tissues normal
6533/F	50 (TK)	13	FndDd	NA	All tissues normal
6542/F	50 (TK)	6	FndDd	NA	All tissues normal
6551/F	50 (TK)	8	FndDd	NA	Al tissues normal
3514/F	100 (Main Study)	10	EuEx	Abdomen distended, Yellow discolored feces, decreased activity, piloerection, breathing audible	No observations found
7054/M	100 (TK)	3	FndDd	NA	All tissues normal
7055/M	100 (TK)	2	FndDd	NA	All tissues normal
7530/F	100 (TK)	13	DAD	NA	All tissues normal
7557/F	100 (TK)	2	FndDd	NA	All tissues normal
4002/M	250 (Main Study)	13	EuEx	Abdomen distended, thin, body cold to touch	No observations found
4012/M	250 (Main Study)	9	EuEx	Abdomen distended, Yellow discolored feces	No observations found
4013/M	250 (Main Study)	8	FndDd	Abdomen distended, Yellow discolored feces	No observations found
4519/F	250 (Main Study)	13	FndDd	Abdomen distended	White discoloration, lung
8536/F	250 (TK)	5	FndDd	NA	All tissues normal

M – Male; F – Female
NA - Not applicable/Not available

EuEx-Euthanized in extremis
FndDd-Found Dead
DAD-Died after dosing

Source: Applicant's report #2939-001/MRX-NC-001.
Abbreviation: TK, toxicokinetic

The cause of three unscheduled male deaths in the main study (Nos. 4002, 4012, and 4013) at 250 mg/kg/day was septicemia, based on microscopic examination (e.g., bacterial colonies in the adrenal gland, kidney, liver, kidney, and spleen).

One main-study female (No. 3514) in the 100 mg/kg/day group was euthanized in extremis, however the cause of moribundity could not be determined from gross and microscopic examination. One main study female (No. 4519) in the 250 mg/kg/day group was found dead, which resulted from a gavage-related injury.

The Applicant stated that the cause of death in the main study and TK groups was not clearly related to administration of maralixibat, based on the minor findings at necropsy and the lack of dose dependency.

Clinical Signs

Maralixibat at all doses produced increases in the incidence of yellow feces and abdominal distension.

Body Weights

On Day 9 and Days 12 to 15, administration of 100 and 250 mg/kg/day produced significant increases in female body weight (up to 10% and 10.5%, respectively).

During Days 7 to 9, all treated males had significant increases in body-weight gain (up to 51.9%, 56.2%, and 68.3% in the 50, 100, and 250 mg/kg/day groups, respectively). Over the entire treatment period (Days 1 to 15), the body-weight gain in the treatment groups was not significantly different from the controls (-1.4%, +9.9%, and +7.8% in the 50, 100, and 250 mg/kg/day groups, respectively).

During Days 7 to 9 and 9 to 10, females in the 100 and 250 mg/kg/day groups had significant increases in body-weight gain (up to 59.7% and 74.1% in the 100 and 250 mg/kg/day groups, respectively). Over the entire treatment period (Days 1 to 15), a significant increase in body-weight gain was observed in the 100 mg/kg/day females (14.7%).

No significant changes in body weight or body-weight gain were observed at the end of the recovery period.

Feed Consumption

Food consumption was evaluated from Day 15 to Day 43 (PND 21 to 49). There were no drug-related effects.

Ophthalmoscopy

Not applicable.

Hematology

Treatment with ≥50 mg/kg/day produced significant increases in red cell indices (23% for red blood cells and 27% for hemoglobin at 250 mg/kg/day in both sexes, 25% for hematocrit at 250 mg/kg/day in females), and absolute reticulocyte count (up to 48.4%). These changes were reversible.

Due to the small sample size (one to three for most groups), the data from coagulation tests (prothrombin time, aPTT, and fibrinogen) were insufficient to allow for a meaningful evaluation at the end of the treatment period. However, no changes were observed at the end of the recovery period.

Clinical Chemistry

Maralixibat at ≥50 mg/kg/day produced significant dose-dependent increases in ALP (up to 91.9%) and phosphorus (up to 16.4% at 100 and 250 mg/kg/day) concentrations. No changes were observed at the end of the recovery period.

Urinalysis

Not applicable.

Sexual Maturation

There were no drug-related effects on preputial separation or vaginal opening.

Reproductive Capacity

Not applicable.

Central Nervous System/Neurobehavioral Assessment

Not applicable.

Bone Evaluation

The right femur was measured from the proximal end of the femoral head to the distal end of the medial condyle and was subjected to bone densitometry evaluation using peripheral quantitative computed tomography. The ex vivo peripheral quantitative computed tomography reported parameters are listed in Table 87.

Table 87. Ex Vivo Peripheral Quantitative Computed Tomography Parameters

Scan Site	Reporting
Femur Metaphysis	Total area (mm^2), BMC (mg/mm) and BMD (mg/cm^3) Trabecular BMC (mg/mm) and BMD (mg/cm^3) Cortical/subcortical BMC (mg/mm) and BMD (mg/cm^3)
Femur Diaphysis	Total area (mm^2), cortical area (mm^2), BMC (mg/mm) and BMD (mg/cm^3) Cortical thickness (mm) Periosteal circumference (mm) Endosteal circumference (mm) Cross-sectional moment of inertia in the plane of bending, CSMI (mm^4)

BMC: Bone Mineral Content; BMD: Bone Mineral Density.
Source: Applicant's report #2939-001/MRX-NC-001.

Bone Length

There were no drug-related changes in femur length in either sex in the main study and recovery groups.

Bone Densitometry—Ex Vivo

Males at 100 and 250 mg/kg/day showed trends for increases in distal femur BMC for the total, trabecular, and cortical/subcortical areas (9.3% to 19.7% at 100 mg/kg/day; 14.1% to 23.1% at 250 mg/kg/day). These changes in bone mass were associated with increases in BMD for the total, trabecular, and cortical/subcortical areas (250 mg/kg only) (5.9% to 14.6%). The increases in total BMD and cortical/subcortical BMD at 250 mg/kg/day (+8.9% and +5.9%, respectively) were statistically significant.

Females at 250 mg/kg/day had a significant increase in distal femur total slice area (15.8%), associated with significant increases in total, trabecular, and cortical/subcortical BMC (19.6% to 43%). These increases in bone size and bone mass were associated with significant increases in total and trabecular BMD (10.1% and 24.2%, respectively).

Gross Pathology

There were no drug-related effects.

Organ Weights

Treatment with 250 mg/kg/day produced a significant increase in absolute and relative spleen weight, as shown in Table 88. This change was reversed in the recovery groups.

Table 88. Summary of Organ Weight Data—Terminal Euthanasia (Day 15)

	Males			Females		
Group	**2**	**3**	**4**	**2**	**3**	**4**
Dose (mg/kg/day)	**50**	**100**	**250**	**50**	**100**	**250**
No. Animals per Group	**10**	**10**	**9**	**10**	**10**	**10**
Spleen (No. Weighed)[a]	10	10	9	10	10	10
Absolute value	-8.3	5.1	**36.5**	2.7	2.8	**32.7**
% of body weight	-7.6	2.0	**21.1**	-0.6	-3.0	**16.7**
% of brain weight	-6.9	6.9	**35.0**	1.8	5.1	**32.1**

[a] All values expressed as percent difference of control group means.

Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

Source: Applicant's report #2939-001/MRX-NC-001.

Histopathology

One of nine males in the 250 mg/kg/day group had marked hyperplasia in the bile duct. Bile duct hyperplasia was not considered as drug-related due to the low incidence. No changes were observed at the end of the recovery period.

Toxicokinetics

With respect to the entire dose range tested (250 mg/kg compared to 50 mg/kg), the increase in $AUC_{0\text{-}24h}$ was markedly lower than the dose increment, suggesting that saturation of absorption occurred at 250 mg/kg. C_{max} values at 50 and 250 mg/kg were similar on Days 1 and 14, with only a slight increase at 250 mg/kg on Day 8. The marked decrease in systemic exposure between PND 7 (Day 1) and PND 20 (Day 14) is typical of the maturation-related decrease in systemic drug exposure that routinely occurs in toxicity studies with neonatal/juvenile rats. This event is primarily due to the high permeability of the immature intestine of neonatal rats. There were no sex-related differences in TK parameters. See Table 89.

Table 89. Toxicokinetic Parameters in an Oral Toxicity Study in Juvenile Rats

Dose (mg/kg/day)	Day	C_{max} (ng/mL)	C_{max}/Dose (kg*ng/mL/mg)	T_{max} (hr)	T_{last} (hr)	AUC_{0-24hr} (hr*ng/mL)	AUC_{0-24hr}/Dose (hr*kg*ng/mL/mg)	R[a]
50	1	961	19.2	5	24	11700	234	NA
50	8	353	7.07	3	24	4170	83.4	0.356
50	14	39.2	0.785	1	24	343	6.87	0.0293
100	1	713	7.13	8	24	13200	132	NA
100	8	422	4.22	8	24	6900	69.0	0.524
100	14	30.3	0.303	1	24	325	3.25	0.0246
250	1	902	3.61	5	24	13900	55.5	NA
250	8	445	1.78	3	24	6430	25.7	0.463
250	14	41.3	0.165	1	24	471	1.89	0.0340

NA - Not applicable.
a: $R = AUC_{0-24hr\ Day\ 8\ or\ Day\ 14}/AUC_{0-24hr\ Day\ 1}$.
Source: Applicant's report #2939-001/MRX-NC-001.
Abbreviations: AUC_{0-24hr}, area under the curve from time 0 to 24 h; C_{max}, maximum concentration; NA, not applicable; T_{max}, time to maximum concentration

13.2.3.3.2. Study Title: LUM001 [Maralixibat]: Preliminary Toxicokinetic and Dose Range Finding Study and 43-Day Toxicity Study via Oral Gavage with Recovery in Juvenile Rat

Key Findings

- Systemic exposure to maralixibat and accumulation following repeated dosing were observed.
- No target organ of toxicity was identified.
- The NOAEL for males was 200 mg/kg/day (highest dose tested), and the NOAEL for females was 1000 mg/kg/day (highest dose tested).

Study no.:	13-4397
Study report location:	Module 4, Nonclinical study reports
Study initiation date:	March 15, 2013
Conducting laboratory and location:	(b) (4)
Duration:	43
Duration units:	days
GLP compliance:	Yes
Drug, lot number, and percentage purity:	AA-025325-Batch-03-2012; 98.81%; AA-025325-Batch-03-2013; 99.61%
Target organ:	Choose an item.

Table 90. Study Information

Methods	
Doses:	See Table 91
Frequency of dosing:	Once daily
Number/sex/group:	See Table 91
Dose volume:	5 mL/kg
Formulation/vehicle:	5, 10, 20, 40 mg/mL in males; 25, 50, 100, 200 mg/mL in females/distilled deionized water
Route of administration:	ORAL GAVAGE
Species:	RAT
Strain:	CD1(ICR)
Age/sexual maturity:	Postnatal day 21
Comment on study design and conduct:	This study included two phases. See study design table for details.
Dosing solution analysis:	Meets all acceptance criteria.

Source: Prepared by the nonclinical reviewer

Biomarker	
Biomarker evaluated:	Choose an item.
If "Other" was selected:	Click here to enter text.
Purpose of biomarker:	Choose an item.

Phase 1A: Rats in the toxicokinetic (TK) study were treated once on postnatal day (PND) 21. Blood was collected from three animals/sex/timepoint on PND 21 at 1, 2, 3, 5, 8, and 24 h postdose for TK analysis. The animals were euthanized and discarded without further examination.
Phase 1B: Rats in the TK study were treated once daily for 8 consecutive days beginning on PND 21. Blood was collected from three animals/sex/timepoint on PND 28 at the estimated time to maximum concentration (3 h postdose). After the TK blood collection, all animals were euthanized, and a macroscopic examination of the gastrointestinal tract was performed.
Phase 2: Rats in the main study were treated once daily for 43 consecutive days beginning on PND 21, followed by a 35-day recovery period.

Table 91. Study Design

Group	Daily Dose[a]					Number of Animals							
						Total on Study		Toxicity				TK[b]	
								Terminal Necropsy		Recovery Necropsy		PND 21/22 (Phase 1A), PND 28 (Phase 1B) or PND 56/57 (Phase 2)	
	Dose (mg/kg)		Vol (mL/kg)	Conc. (mg/mL)									
	M	F		M	F	M	F	M	F	M	F	M	F
Phase 1A (Toxicokinetics)													
1	0	0	5	0	0	18	18	-	-	-	-	18	18
2	25	125	5	5	25	18	18	-	-	-	-	18	18
3	50	250	5	10	50	18	18	-	-	-	-	18	18
4	100	500	5	20	100	18	18	-	-	-	-	18	18
5	200	1000	5	40	200	18	18	-	-	-	-	18	18
Phase 1B (Dose Range Finding)													
1	0	0	5	0	0	5	5	5	5	-	-	3	3
2	25	125	5	5	25	5	5	5	5	-	-	3	3
3	50	250	5	10	50	5	5	5	5	-	-	3	3
4	100	500	5	20	100	5	5	5	5	-	-	3	3
5	200	1000	5	40	200	5	5	5	5	-	-	3	3
Phase 2 (Main Study)													
1	0	0	5	0	0	18	18	12	12	6	6	12	12
2	50	250	5	10	50	18	18	12	12	6	6	12	12
3	100	500	5	20	100	18	18	12	12	6	6	12	12
4	200	1000	5	40	200	18	18	12	12	6	6	12	12

[a] The test article is LUM001; doses are expressed in terms of LUM001 free base equivalent. Purity was assumed to be 100% for dose calculation.
[b] Toxicokinetics
For all study phases (Phase 1A, 1B and 2) the first day of dosing was defined as PND 21

Source: Applicant's report #13-4397.
LUM001: code name for maralixibat
Abbreviations: conc, concentration F, female; M, male; PND, postnatal day; TK, toxicokinetic; vol, volume

Observations and Results

Mortality

One female in the 250 mg/kg/day group that was assigned to Phase 1A and 1B was found dead on PND 21. No macroscopic or microscopic examination was performed. The Applicant stated that the cause of death was not drug-related.

There were nine unscheduled deaths in the Phase 2 study, none of which was considered to be drug-related. Two males in the 50 mg/kg/day group and two males in the 100 mg/kg/day group were found dead on PNDs 35, 30, 23, and 23, respectively. Two females in the 250 mg/kg/day group, one female in the 500 mg/kg/day group, and one female in the 1000 mg/kg/day group were found dead on PNDs 33, 29, 23, and 32, respectively. One female in the 1000 mg/kg/day group was sacrificed due to moribund condition on PND 60. Macroscopic and microscopic findings indicated that gavage-related injury was the likely cause of these deaths.

Clinical Signs

There were no drug-related clinical signs.

Body Weights

Phase 1B

Body weights in the 500 mg/kg/day female group were reduced by 10.8% at the end of the study (PND 28), as compared to the controls.

Phase 2

There were no effects on body weight or body-weight gain.

Feed Consumption

There were no effects on food consumption in males.

Maralixibat at 500 and 1000 mg/kg/day produced significant increases in food consumption in females (up to 20%) during the following treatment intervals: PND 24 to 28, PND 28 to 31, PND 38 to 42, PND 45 to 49, PND 49 to 52 (1000 mg/kg/day only), PND 52 to 55, PND 55 to 59, and PND 59 to 63. No changes were observed at the end of the recovery period.

Ophthalmoscopy

There were no drug-related ophthalmological findings.

Electrocardiogram

Not applicable.

Hematology

Maralixibat at all doses produced a dose-dependent and significant decrease in prothrombin time in males, which was not reversible at the end of the recovery period. In addition, aPTT was reduced by up to 22% in all males at the end of the recovery period ($p \leq 0.05$) without dose-dependency.

In females, maralixibat at all doses produced slight but significant decreases in prothrombin time (up to 6.5%). maralixibat at 1000 mg/kg/day in females produced a significant increase in aPTT (up to 14.5%). These changes were not reversible. The increase in aPTT was likely related to vitamin K deficiency due to the pharmacological activity of the test article, whereby the blockade of bile acid reabsorption and the resulting depletion of bile acids reduced FSV absorption.

Clinical Chemistry

Males in the 200 mg/kg/day group had a 22% increase in ALP ($p \leq 0.01$). No change was observed at the end of the recovery period.

Urinalysis

Not applicable.

Gross Pathology

There were no treatment-related macroscopic changes in the Phase 1B and Phase 2 studies. All macroscopic changes were observed in animals found dead. The cause of death was determined to be gavage-related injury based on the observed presence of foreign material (test article) in bronchioles and/or alveoli in eight of nine unscheduled deaths. Red or white foamy fluid in the tracheal lumen was present in six of nine unscheduled deaths. This finding correlated microscopically with intraluminal exudate in the trachea of one moribund female. Four of the unscheduled deaths had moderate to severe lung distension, and red discoloration.

Organ Weights

Maralixibat at all doses produced significant decreases in absolute adrenal gland weight (↓up to 13%) in males, without dose-dependency. Maralixibat at 200 mg/kg/day produced significant decreases in adrenal gland weight relative to body weight (11%) and brain weight (14%) in males. The changes were not reversible.

Maralixibat at all doses produced a significant decrease in absolute epididymis weight (up to 9%), without dose-dependency. There was a significant decrease in epididymis weight relative to body weight (↓9%) in the 200 mg/kg/day group, and a dose-dependent, significant decrease in epididymis weight relative to brain weight was observed (↓ up to 10%). No change in epididymis weight was observed at the end of the recovery period.

Maralixibat at all doses produced significant, non-dose-dependent increases in absolute ovary weight (↑up to 32%). There was a significant increase in ovary weight relative to body weight (↑25%) in the 1000 mg/kg/day group, and a significant, non-dose-dependent increase in ovary weight relative to brain weight was observed (↑ up to 34%). No change in ovary weight was observed at the end of the recovery period.

Maralixibat at all doses produced significant, non-dose-dependent increases in absolute liver weight (↑up to 14%) and relative liver weight (relative to brain weight: ↑up to 15%; relative to body weight: ↑up to 8%) in females. No changes in liver weight were observed at the end of the recovery period.

Histopathology

Adequate battery: Yes

Peer review: No

There were no drug-related microscopic changes in animals in the Phase 1B and Phase 2 studies.

All microscopic changes were observed in the lungs of animals that were found dead, including alveolar necrosis, hemorrhage/congestion, and foreign material and/or edema with fibrin.

Special Evaluation

Motor Activity, Functional Observational Battery Evaluation, and Sexual Maturation

There were no drug-related changes in motor activity (horizontal and vertical movement) or Functional Observational Battery, with the exception of statistically significant decreases in landing foot splay measurements in all females treated with maralixibat (up to 15.4%). The decreased landing foot splay was not dose-dependent and there were no changes in grip strength (forelimb and hindlimb), gait, or locomotor activity. No changes were observed at the end of the recovery period.

Maralixibat at all doses had no effects on sexual maturation in males (preputial separation) or females (vaginal opening).

Toxicokinetics

Systemic exposure (C_{max} and AUC_{0-24h}) to maralixibat in juvenile rats to tended to increase with dose. However, the increases were inconsistent due to a small number of samples in which markedly higher concentrations of maralixibat were found, compared to the samples taken at or about the same time from other animals in the same treatment group.

In general, the systemic exposure increased in a less than dose-proportional manner, although the AUC_{0-24h} values in females increased in a greater than dose-proportional manner at 500 to 1000 mg/kg/day. The accumulation ratios were close to or less than one in males, but were greater than one in females, indicating that accumulation only occurred in female rats after repeated oral administration.

The TK parameters are summarized in Table 92, Table 93, Table 94, and Table 95.

Table 92. Toxicokinetic Parameters in Plasma After Oral Administration of a Single Dose of Maralixibat in Juvenile Rats on Postnatal Day 21 (Phase 1A)

Postnatal Day 21 (Phase 1A)					
Males			Females		
Dose level (mg/kg)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)	Dose level (mg/kg)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)
25	4.27	51.4	125	63.6	321
50	52.2	202	250	30.4	265
100	21.1	151	500	50.4	566
200	23.7	280	1000	258	1400

Source: Applicant's report #13-4397.
Abbreviations: AUC_{0-24}, area under the curve from time 0 to 24 h; C_{max}, maximum concentration

Table 93. Mean Plasma Concentrations of Maralixibat After 8 Days of Oral Dosing (PND 28), 3 h Postdose (C_3) in Juvenile Rats (Phase 1B)

Postnatal Day 28 (Phase 1B)			
Males		Females	
Dose level (mg/kg/day)	C_3 (ng/mL)	Dose level (mg/kg/day)	C_3 (ng/mL)
25	1.58	125	13.3
50	2.70	250	8.07
100	5.17	500	20.4
200	6.40	1000	109

Source: Applicant's report #13-4397.
Abbreviation: PND, postnatal day

Table 94. Toxicokinetic Parameters in Plasma on Day 35 of Oral Administration of Maralixibat in Juvenile Rats (PND 56, Phase 2)

Postnatal Day 56 (Phase 2)					
Males			Females		
Dose level (mg/kg/day)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)	Dose level (mg/kg/day)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)
50	6.86	43.8	250	176	413
100	14.0	140	500	76.1	939
200	13.0	133	1000	370	2570

Source: Applicant's report #13-4397.
Abbreviation: AUC_{0-24}, area under the curve from time 0 to 24 h; C_{max}, maximum concentration; PND, postnatal day

Table 95. Changes in Exposure (Accumulation Ratios) Following Repeated Administration of Maralixibat During Phase 2

Dose level (mg/kg/day)		Accumulation ratio	
Males	Females	Males	Females
50	250	0.22	1.6
100	500	0.93	1.7
200	1000	0.48	1.8

Source: Applicant's report #13-4397.

13.2.3.4. Study Title: A 26-Week Oral Gavage Carcinogenicity (001178-T [Hemizygous]) and Toxicokinetic (001178-W [Wild-Type]) Study with Maralixibat in RasH2 Mice

Key Study Findings

Study no.:	MRXNC-002
Study initiation date:	June 11, 2019
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot number, and percentage purity:	Maralixibat/ #CA16-1118/ 99.8% N-methyl-N-nitrosourea/ #14-MWC-167-1/ 95%
Prior ECAC dose concurrence:	Yes
Basis for dose selection:	In a 4-week oral gavage dose range-finding study in RasH2 (wild-type) mice (Study #M10806M-SHP625, conducted by (b) (4)), the maximum tolerated dose (MTD) in males was identified at 75 mg/kg/day by the nonclinical reviewer, based on a significant reduction in body-weight gain and hypertrophy of renal tubule cells at 250 mg/kg/day. The MTD in females was considered to be 250 mg/kg/day by the reviewer. The ECAC recommended doses of 0, 2.5, 7.5, and 25 mg/kg/day for males. The high dose was based on the adverse effect on body-weight gain at 75 mg/kg/day in the 4-week dose range-finding study. For females, the ECAC recommended doses of 0, 7.5, 25, and 75 mg/kg/day. The high dose was based on the adverse effect on body-weight gain at 250 mg/kg/day in the 4-week dose range-finding study.

Reviewer's carcinogenicity conclusion (negative/positive): Negative

Executive Carcinogenicity Assessment Committee (ECAC) Carcinogenicity conclusion (negative/positive): Negative

Tumor Findings

Male and female CB6F1-TgN (RasH2) transgenic mice were administered maralixibat via oral gavage at daily doses of 0, 2.5, 7.5 and 25 mg/kg in males and 0, 7.5, 25 and 75 mg/kg in females for 26 weeks. The vehicle control group received deionized water. The design and doses for this study were approved by the ECAC on May 7, 2019.

The dosing of male mice at 25 mg/kg/day was associated with increased mortality but the number of surviving animals (20 of 25) allowed for statistical evaluation of tumor incidence.

No clear drug-related increase in neoplastic findings was noted after exposure to doses up to 75 mg/kg/day. Increased incidences of hemolymphoreticular malignant lymphoma, squamous cell skin papilloma, and squamous cell stomach papilloma were observed in N-methyl-N-nitrosourea (MNU)-treated transgenic mice, consistent with previous studies.

The ECAC concluded that the carcinogenicity study was adequate, noting prior approval of the protocol, and that no drug-related neoplasms were observed in either males or females (ECAC meeting held on June 15, 2021).

Table 96. Study Information

Methods	
Doses:	Males: 0 (deionized water), 2.5, 7.5, 25 mg/kg/day Females: 0 (deionized water), 7.5, 25, 75 mg/kg/day MNU (positive control): 75 mg/kg IP once (Day 1)
Frequency of dosing:	Once daily
Number/sex/group:	25 (test article and vehicle control article) 10 (positive control)
Dose volume:	5 mL/kg
Formulation/vehicle:	solution/deionized water
Route of administration:	ORAL GAVAGE
Species:	MOUSE
Strain:	CB6F1-TgN (RasH2)
Age:	8-9 weeks
Comment on study design and conduct:	The study included satellite groups for toxicokinetics (36/sex/test article group and 6/sex for vehicle). The positive control (MNU) was administered intraperitoneally in acidified physiological saline (150mM sodium chloride and 15mM sodium citrate in reverse-osmosis water adjusted to pH 4.5.
Dosing comments:	None
Dosing solution analysis:	All formulations met the specifications of homogeneity and target concentrations, except for the Week 26 formulation with 15 mg/mL as the nominal concentration (76% of nominal). From Days 165 to 178, the high-dose females received a formulation with a lower concentration that delivered 57 mg/kg/day instead of 75 mg/kg/day, whereas the same group received the proper dose prior to this period. Based on the Week-26 toxicokinetic data, the Applicant did not anticipate an impact on the overall interpretation of the tumor findings.

Abbreviations: IP, intraperitoneally; MNU, N-methyl-N-nitrosourea

Observations and Results

Mortality

Totals of six males, six females, and one TK female were necropsied/found dead at an unscheduled interval during the study. The sex, dose, date of death, and clinical observations noted prior to the event and the reported cause of death are presented in Table 97.

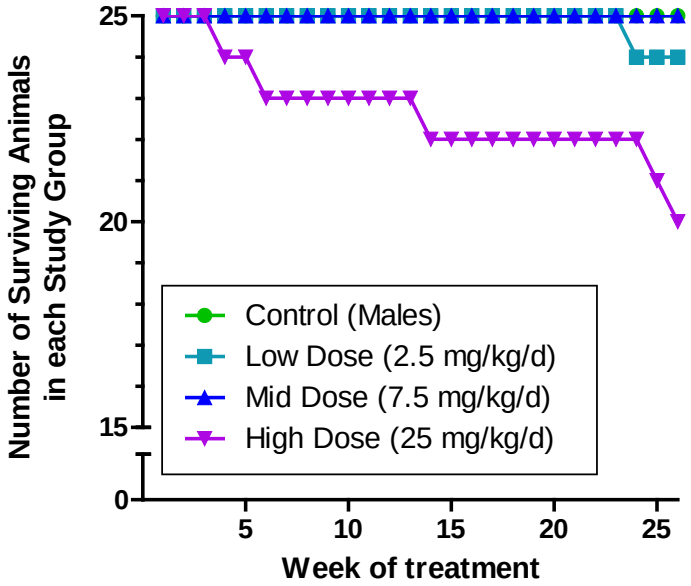
Table 97. Mortality, Study MRXNC-002

Group/ Sex	Animal Number	Maralixibat (mg/kg/day)	Study Day of Death/Sacrifice	Relevant Clinical Observations	Cause of Death
2/M	M0104	2.5	164	Decreased general activity, hunched posture, whole body cool to touch, abnormal/pale feet	Undetermined
4/M	M0301	25	42	Piloerection, hunched posture, labored respiration, whole body cool to touch	Undetermined
4/M	M0304	25	96	Piloerection, hunched posture, low carriage, irregular respiration, whole body cool to touch	Undetermined
4/M	M0319	25	170	Piloerection, thin appearance, hunched posture, abnormal tail color, with palpable mass	Hemangiosarcoma, skin/subcutis
4/M	M0322	25	26	found dead	Hemangiosarcoma, Spleen
4/M	M0325	25	182	Liquid feces, piloerection, hunched posture	Undetermined
2/F	M0602	7.5	172	Whole body pale, decreased general activity, swollen shoulder area, thin appearance, hunched posture, whole body cool to touch	Malignant Lymphoma
2/F	M0616	7.5	137	Found dead	Undetermined
2/F	M0619	7.5	29	Deep injury at the base of tail, sacrificed due to location of tail injury	Undetermined
3/F	M0707	25	100	Decreased general activity, hunched posture, abnormal skin and feet color	Undetermined
3/F	M0715	25	120	Hunched posture	Undetermined
4/F	M0808	75	120	No remarkable observations	Urogenital infection/inflammation

Source: Applicant's report #MRXNC-002.
Abbreviations: F, female; M, male

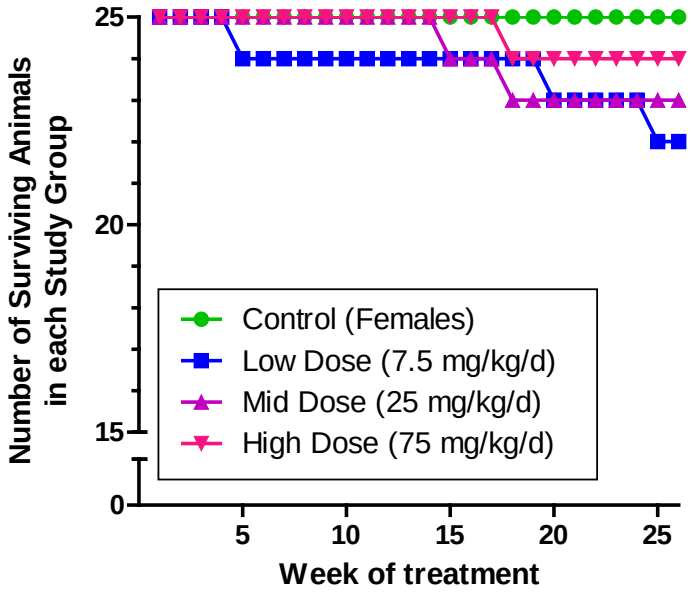
All other animals survived until the end of the study and terminal group numbers were sufficient for the statistical evaluation of a potential carcinogenic effect. Nonetheless, dosing of maralixibat was associated with a dose-dependent, statistically significant reduction in survival of male mice (p=0.0045). The high-dose males (25 mg/kg/ day) had significantly higher (p=0.0225) premature deaths than the control group. This effect was not observed in the female groups at doses of up to 75 mg/kg/day. The positive control article (MNU) significantly decreased the survival rate of Tg-RasH2 mice of both sexes after a single intraperitoneal injection of 75 mg/kg. See Figure 14 and Figure 15.

Figure 14. Survival Over Time of Male Tg.RasH2 Mice



Source: Prepared by the nonclinical reviewer.

Figure 15. Survival Over Time of Female Tg.RasH2 Mice



Source: Prepared by the nonclinical reviewer.

Clinical Signs

The clinical observations were generally consistent with those reported in the 4-week range-finding study and consisted of decreased general activity, piloerection, thin appearance, hunched posture, and irregular respiration.

Body Weights

At the end of the dosing period, minor decreases in mean absolute body weights (up to 2.4 g) compared to controls were noted in males administered ≥7.5 mg/kg/day, whereas no differences in mean body weights occurred in females. These results indicate that the high dose in males was likely the maximum tolerated dose. See Table 98.

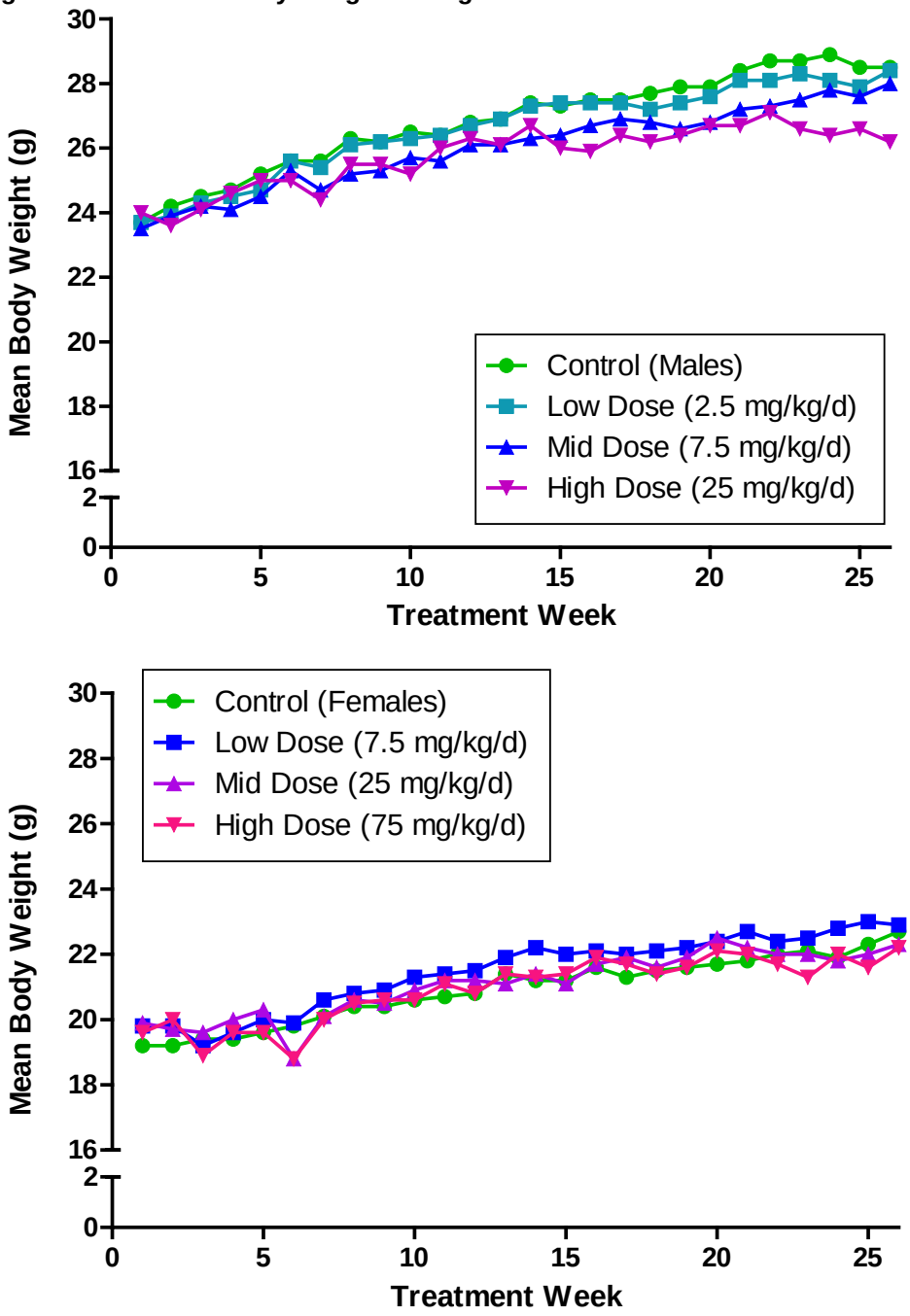
Table 98. Changes in Mean Absolute Body Weights

	Males				Females			
		Maralixibat (mg/kg/day)				Maralixibat (mg/kg/day)		
Day 182	**Control**	**2.5**	**7.5**	**25**	**Control**	**7.5**	**25**	**75**
Number	25	24	25	20	25	22	23	24
BW (g)	28.9	28.4	27.2	26.5	22.1	22.7	21.8	22.1
SD (g)	±2.06	±2.67	±2.33	±2.24	±1.62	±1.49	±1.37	±1.12
Change in BW relative to controls	-	-1.86%	-5.80%	-8.19%	-	2.99%	-1.06%	0.11%

Source: Prepared by the nonclinical reviewer.
Abbreviations: BW, body weight; SD, standard deviation

The absolute body-weight changes in male and female mice are presented in Figure 16.

Figure 16. Absolute Body-Weight Changes in Male and Female Mice



Source: Prepared by the nonclinical reviewer.

Feed Consumption

No treatment-related changes in food consumption were noted at the end of the study.

Gross Pathology and Organ Weights

At the terminal sacrifice, decreased spleen weights were noted only in the high-dose females (75 mg/kg/day), with no macroscopic or microscopic correlates. All other differences in organ

weight parameters were consistent with normal variation and considered incidental. No treatment-related macroscopic observations were noted.

Histopathology

Peer review conducted: Yes

Historical control provided for tumor incidence: No. However, the reviewer retrieved information from published literature to support the data analysis (Paranjpe et al. 2013a).

Neoplastic

The Applicant reported increases in the incidence of bronchiolo-alveolar adenoma and carcinoma in males administered 25 mg/kg/day maralixibat. Bronchiolo-alveolar adenomas were statistically significant in the pairwise and trend tests in the Applicant's statistical analysis, as was the combined incidence of bronchiolo-alveolar adenoma and bronchiolo-alveolar carcinoma.

The tumors observed in the study are presented in Table 99.

Table 99. Incidence of Neoplastic Findings Following 26 Weeks of Maralixibat Administration in RasH2 Mice

	Male (mg/kg/day)					Female (mg/kg/day)				
Organ/Tissue Finding	**0**	**2.5**	**7.5**	**25**	**MNU 75**	**0**	**7.5**	**25**	**75**	**MNU 75**
Hemolymphoreticular tissue										
Lymphoma, malignant					8		1			9
Stomach, nonglandular										
Squamous cell carcinoma, malignant					1					
Squamous cell papilloma, benign					7			1		3
Thymus										
Hemolymphoreticular tumor, malignant					8					7
Thymoma, benign	2	1								
Lung										
Bronchiolo-alveolar adenoma, benign	1	2		6			1	1		
Bronchiolo-alveolar carcinoma, malignant				1						
Hemolymphoreticular tumor, malignant										1
Skin, subcutis										
Hemangiosarcoma, malignant				1						
Hemolymphoreticular tumor, malignant										1
Squamous cell carcinoma, malignant					1					3
Squamous cell papilloma, benign					6					
Spleen										
Hemangioma, benign								1		
Hemangiosarcoma, malignant		1	1	1					1	
Hemolymphoreticular tumor, malignant					6		1			7
Lymph nodes										
Hemolymphoreticular tumor, malignant					4					6
Liver										
Hemolymphoreticular tumor, malignant					1		1			3
Lymph node mandibular										
Hemolymphoreticular tumor, malignant					2					3
Harderian gland										
Adenoma, benign		1				2	1		1	
Lymph node, mesenteric										
Hemolymphoreticular tumor, malignant					2					2

	Male (mg/kg/day)					Female (mg/kg/day)				
Organ/Tissue Finding	**0**	**2.5**	**7.5**	**25**	**MNU 75**	**0**	**7.5**	**25**	**75**	**MNU 75**
Uterus										
Hemangioma, benign									1	1
Hemolymphoreticular tumor, malignant										1
Kidney										
Hemangioma, benign			1							
Ovary										
Hemolymphoreticular tumor, malignant										1
Hemangioma, benign									1	
Testis										
Leydig cell tumor, benign					1					

Source: Prepared by the nonclinical reviewer.
Abbreviation: MNU, N-methyl-N-nitrosourea

The tumor groupings used for the FDA statistical analysis are listed in Table 100.

Table 100. Tumor Groups Used for the FDA Statistical Analysis

Males	Females
Lungs: Bronchiolo-alveolar adenoma + bronchioloalveolar carcinoma	All tissues: Hemangioma
All tissues: Hemangiosarcoma	All tissues: Hemangioma + hemangiosarcoma
All tissues: Hemangioma + hemangiosarcoma	

Source: Prepared by the nonclinical reviewer.

The statistical reviewer, Dr. Zhuang Miao, analyzed the tumor data using the trend test for dose-response relationship and pairwise comparisons between the vehicle control and three treatment groups.

From these comparisons, the following statistical conclusions were made (summarized from the Statistical Review and Evaluation dated 07/28/2021):

- For male mice, the tumor data analysis showed a statistically significant positive dose-response relationship for the incidence of lung bronchiolo-alveolar adenoma ($p=0.0036<0.05$) and for the combined incidence of bronchiolo-alveolar adenoma and carcinoma ($p<0.001$) in the treatment group. The comparison between the vehicle control and the high-dose males showed statistically significant increases in the incidence of bronchiolo-alveolar adenoma ($p=0.0324<0.05$) and the combined incidence of bronchiolo-alveolar adenoma and carcinoma ($p=0.0146<0.05$).
- For female mice, the tumor data analysis showed a statistically significant positive dose-response relationship for the combined incidence of hemangiosarcoma + hemangioma ($p=0.0196<0.05$).
- For male mice, comparison of the vehicle control and positive control groups showed statistically significant increases in the incidence of lymphoma, hemolymphoreticular tumors ($p<0.001$), squamous cell papilloma in skin/subcutis ($p<0.001$), and squamous cell papilloma in stomach ($p<0.001$).
- For female mice, the comparison between the vehicle control and the positive control group showed statistically significant increase in the incidence of lymphoma, hemolymphoreticular tumor ($p<0.001$), squamous cell carcinoma in skin/subcutis ($p=0.0025<0.05$), and squamous cell papilloma in stomach ($p=0.0025<0.05$).

Table 101and Table 102 show the calculated p-values for the trend test and pairwise comparisons for bronchiolo-alveolar tumors in males and hemangiomas and hemangiosarcomas in females.

Table 101. Tumor Types with P-Values ≤0.05 for Comparisons Between Vehicle Control and Treated Groups in Male Mice

Organ	Tumor Name	0 mg Control (N=25) P, Trend	2.5 mg/kg Low (N=25) P, C vs. L	7.5 mg/kg Med (N=25) P, C vs. M	25 mg/kg High (N=25) P, C vs. H
Lung	Bronchiolo-alveolar adenoma	1/25 (25) 0.0036	2/25 (25) 0.5000	0/25 (25) 1.0000	6/25 (22) 0.0324
	Bronchiolo-alveolar adenoma + carcinoma	1/25 (25) <0.001	2/25 (25) 0.5000	0/25 (25) 1.0000	7/25 (22) 0.0146

Source: Adapted from the Statistical Review and Evaluation by Dr. Zhuang Miao dated 07/28/2021.
X/ZZ (YY): X=number of tumor-bearing animals; YY=mortality-weighted total number of animals; ZZ=unweighted total number of animals observed.
Abbreviations: C, control; H, high; L, low

Table 102. Tumor Types with P-Values ≤0.05 for Comparisons Between Vehicle Control and Treated Groups in Female Mice

Organ	Tumor Name	0 mg Control (N=25) P, Trend	7.5 mg/kg Low (N=25) P, C vs. L	25 mg/kg Med (N=25) P, C vs. M	75 mg/kg High (N=25) P, C vs. H
Whole body	Hemangiosarcoma + hemangioma	0/25 (25) 0.0196	0/25 (23) NC	1/25 (24) 0.4898	3/26 (25) 0.1173

Source: Adapted from the Statistical Review and Evaluation by Dr. Zhuang Miao dated 07/28/2021.
XX/ZZ (YY): X=number of tumor-bearing animals; YY=mortality-weighted total number of animals; ZZ=unweighted total number of animals observed.
Abbreviations: C, control; H, high; L, low

Bronchial adenomas and carcinomas of the lungs were the most common spontaneous tumors in Tg.rasH2 mice, with a combined incidence of 12.25% in males and 7.74% in females. Therefore, these tumors should be evaluated as common tumors in the statistical analysis. In accordance with ECAC policy, the significance levels for the trend test and pairwise test for common tumors should be 0.005 and 0.01, respectively, which differ from those used by the statistical reviewer (0.05 for both the trend and pairwise tests). The p-value for the trend test was significant for bronchiolo-alveolar adenoma ($p=0.0036<0.005$) and for the combination of bronchiolo-alveolar adenoma and carcinoma ($p<0.001<0.005$) in males. However, the pairwise test did not show significance for bronchiolo-alveolar adenoma ($p=0.0324>0.01$) or the combination of bronchiolo-alveolar adenoma and carcinoma ($p=0.0146>0.01$). Hence, the data did not conclusively demonstrate a statistically significant increase in the incidence of bronchiolo-alveolar adenomas and/or carcinomas after 26 weeks of maralixibat administration in Tg-RasH2 mice.

Hemangiomas and hemangiosarcomas are the second most common spontaneous tumors in Tg-RasH2 mice. In females, neither the trend test nor any of the pairwise comparisons reached the level of statistical significance for common tumors.

As expected, the positive control article, MNU, produced a highly significant increase in the incidence of lymphoma, squamous cell carcinomas, and stomach papilloma in both male and female Tg-RasH2 mice as compared to the vehicle control group, thus demonstrating the sensitivity of the test system. See Table 103.

Table 103. Tumor Types with P-Values ≤0.05 for Comparisons Between Vehicle Control and Positive Control Tg-RasH2 Mice

Organ Name	Tumor Name	Vehicle	Male P-value, Vehicle vs. Positive	Female P-value, Vehicle vs. Positive
Hemolymphoreticular	Malignant lymphoma	0/25 (25)	8/10 (9), <0.001	9/10 (9), <0.001
Skin/subcutis	Squamous cell papilloma	0/25 (25)	6/10 (7), <0.001	3/10 (5), 0.0025
Nonglandular stomach	Squamous cell papilloma	0/25 (25)	7/10 (8), <0.001	3/10 (5), 0.0025

Source: Adapted from the Statistical Review and Evaluation by Dr. Zhuang Miao dated 07/28/2021.
XX/ZZ (YY): X=number of tumor-bearing animals; YY=mortality-weighted total number of animals; ZZ=unweighted total number of animals observed.

Non-Neoplastic

A microscopic finding of degenerative myopathy of the biceps femoris muscle in animals from all groups, including the vehicle controls, was consistent with the spontaneously occurring skeletal muscle myopathy reported for this mouse strain (Paranjpe et al. 2013b). This expected muscle change, and all other microscopic non-neoplastic changes, were not dose-dependent.

Toxicokinetics

Plasma drug levels were highly variable between individual mice, which may be related to the low oral bioavailability of maralixibat. Plasma drug concentrations generally increased with dose level. After repeated dosing (Week 26), plasma drug levels were higher in males and generally similar in females as compared to day 1 of dosing.

The increases in C_{max} and AUC_{0-24h} values for males were roughly dose-proportional from 2.5 to 25 mg/kg/day on Day 1 and greater than dose-proportional from 7.5 to 25 mg/kg/day at Week 26. The increases in C_{max} and AUC_{0-24h} values for females were generally less than dose-proportional from 7.5 to 75 mg/kg/day on Day 1 and Week 26.

No obvious sex-related differences were observed during Week 26 at 7.5 mg/kg/day.

The C_{max} and AUC_{0-24h} values for maralixibat were generally similar on Day 1 and Week 26 in females, indicating that no drug accumulation occurred after multiple doses of up to 75 mg/kg/day in female mice. Variability in the plasma drug concentrations and the inability to measure predose levels in some animals on Week 26 confound the estimation of drug accumulation in males. The TK parameters are summarized in Table 104.

Table 104. Summary of Maralixibat Toxicokinetic Parameters Following 26 Weeks of Oral Administration to CB6F1-TgN (RasH2) Mice

Interval	Dose Level (mg/kg/day)	Dose Group	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24} (h*ng/mL)
Day 1	2.5	2	M	1.74	2.00	5.92
	7.5	3	M	3.30	2.00	19.2
		2	F	20.9	4.00	109
	25	4	M	9.80	2.00	54.5
		3	F	35.3	4.00	183
	75	4	F	54.8	2.00	399
Week 26	2.5	2	M	59.7	1.00	231
	7.5	3	M	28.2	1.00	75.3
		2	F	26.6	2.00	76.3
	25	4	M	429	2.00	1490
		3	F	55.3	2.00	219
	75[a]	4	F	31.9	2.00	400

NR = Not reported due to inability to characterize the elimination phase.
a Group 4 females received 57 mg/kg/day in error from Days 165 to 178.

Source: Applicant's report #MRXNC-002.
Abbreviations: AUC_{0-24h}, area under the concentration-time curve from time 0 to 24 h; C_{max}, maximum plasma concentration; T_{max}, time to maximum concentration

13.2.3.5. Comments on Inactive Ingredients

Maralixibat oral solution (Livmarli) contains 9.5 mg of maralixibat free-base equivalent per 1 mL (10 mg maralixibat chloride/mL) and the following inactive ingredients: purified water, propylene glycol, disodium ethylenediaminetetraacetic acid dihydrate, sucralose, and grape flavor (b) (4). The proposed maximum daily dose for maralixibat is 3 mL/day (28.5 mg/day), for patients weighing ≥70 kg. Based on the dosing instructions, the dose volume to be administered across all bodyweight bands is approximately 0.04 mL/kg/day. Table 105 lists the

maximum daily doses of inactive ingredients expressed as mg/day (based on administration of 3 mL/day) and mg/kg/day (based on administration of 0.04 mL/kg/day).

Table 105. Maximum Daily Doses of Inactive Ingredients

Inactive Ingredient	Quantity (mg/mL)	Maximum Dose (mg/day)	Maximum Dose (mg/kg/day)
Propylene glycol Disodium EDTA dihydrate Sucralose Grape flavor (b) (4)			(b) (4)

Source: Prepared by the nonclinical reviewer.
Abbreviation: EDTA, ethylenediaminetetraacetic acid

Propylene Glycol

The target population includes patients age 1 to 4 years, in whom propylene glycol can accumulate to toxic levels due to a limited metabolic capacity (via alcohol dehydrogenase). The World Health Organization recommends that the maximum oral daily intake of propylene glycol in neonates and infants not exceed 25 mg/kg/day (assessment report: *Propylene Glycol in Medicinal Products for Children*; European Medicines Agency; Committee for Medicinal Products for Human Use; March 20, 2014). Since the maximum daily dose of propylene glycol in the drug product (b) (4) mg/kg) is lower than the recommended limit for neonates and infants, the amount of propylene glycol in maralixibat is not a safety concern for patients age 1 to 4 years. In addition, the maximum daily dose of (b) (4) mg/day for patients weighing ≥70 kg is not a safety concern, since this dose is lower than the maximum amount of propylene glycol taken orally in other approved drug products.

Sucralose

The maximum daily dose of sucralose delivered in LIVMARLI is (b) (4) mg/kg, which is a small fraction of the acceptable daily dose of sucralose in foods as recommended by the FDA (5 mg/kg, Additional Information about High-Intensity Sweeteners Permitted for Use in Food in the United States, https://www.fda.gov/food/food-additives-petitions/additionalinformation-about-high-intensity-sweeteners-permitted-use-foodunited-states; February 8, 2018). Thus, there is no safety concern for the daily dose of sucralose in LIVMARLI.

Grape Flavor (b) (4)

Grape flavor (b) (4) has been accepted for use in FDA-approved drug products with pediatric indications. The pediatric dose levels (mg/kg/day) of grape flavor in the approved drug products exceed the dose delivered by LIVMARLI. In addition, the approved pediatric drug formulations containing grape flavor (b) (4) include products intended for chronic use. Therefore, the prior accepted use of this grape flavor in drug products for the pediatric population provides a reasonable assurance of safety for the daily dose and duration of exposure with LIVMARLI.

Disodium Ethylenediaminetetraacetic Acid Dihydrate

The maximum daily dose of disodium ethylenediaminetetraacetic acid dehydrate in LIVMARLI is (b) (4) mg/day (patients weighing ≥70 kg), which is lower than the maximum daily dose in other approved drug products for oral administration. Therefore, there is no safety concern for the daily dose of this inactive ingredient in LIVMARLI.

13.2.3.6. Toxicological Risk Assessment of Impurities in Drug Substance and Drug Product

The drug substance contains five impurities that have been observed above the International Council for Harmonisation (ICH) Q3A(R2) identification threshold at release or on stability in relevant batches. These impurities will be controlled as specified nongenotoxic impurities in the drug substance specification.

The Applicant used quantitative structure-activity relationship ((Q)SAR) analysis to evaluate the potential mutagenicity of these impurities and submitted all data in the following study reports:

- Computational evaluation of the toxicity of [redacted] / September 9, 2015).
- In Silico Toxicology Consultancy Report: Toxicological analysis of 24 structures using Derek Nexus and Leadscope ([redacted] / July 18, 2017).
- Computational Mutagenicity Analysis of [redacted] Related Substances (Report # [redacted] 20-141; [redacted] / January 20, 2021]).

The bacterial mutagenicity of these impurities in drug substance was evaluated by (Q)SAR using the Derek Nexus and Leadscope Model Applier Systems. An expert review of the in silico data were submitted by the Applicant and was reviewed by the nonclinical reviewer. All the impurities [redacted] were predicted to be negative in the Derek Nexus analysis. However, in Leadscope Model Applier Systems, the chemical structures of these impurities were not in the domain; therefore, no predictions were made. The Applicant's (Q)SAR analysis is summarized in Table 106.

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To confirm the Applicant's prediction of negative bacterial mutagenicity for the five impurities stated above, a consult request was sent to the Center for Drug Evaluation and Research/Office of Translational Science/Office of Clinical Pharmacology/Division of Applied Regulatory Science Computational Toxicology Consultation Service. The consult also included the drug product impurity (b) (4), which is specified in the drug product specifications (i.e., controlled as a nongenotoxic impurity in accordance with ICH Q3B(R2)). The Computational Toxicology Consultation Service evaluated the potential for bacterial mutagenicity using three (Q)SAR software packages: Derek Nexus version 6.1.0, Leadscope Model Applier version 3.0.2-4 Bacterial Mut version 2 model, and CASE Ultra version 1.8.0.2 GT1_BMUT model. The Center for Drug Evaluation and Research Computational Toxicology Consultation Service concluded that the six impurities are predicted to be negative for bacterial mutagenicity using the Derek Nexus version 6.1.0 and CASE Ultra version 1.8.0.2 GT1_BMUT models (report dated June 9, 2021). The Leadscope Model Applier version 3.0.2-4 Bacterial Mut version 2 model could not generate a prediction for these impurities, except for (b) (4), due to a lack of globally similar structures in the model training set. All chemicals contain an unclassified feature, which is a substructure that is not present in the model reference set. However, the unclassified feature is shared with the active pharmaceutical ingredient (maralixibat), which was negative in the Ames test, thereby supporting a negative prediction. Based on the ICH M7(R1) guidance, these impurities are considered as Class 5 and may be controlled as nonmutagenic impurities.

Justification of Specification of (b) (4)

The justification of specification of four impurities in drug substance is based on a 13-week oral toxicity study in rats and a 43-day oral toxicity study in juvenile rats. The impurities (b) (4) were present in the drug substance batches used in the two nonclinical studies. The proposed acceptance limits for (b) (4) in drug substance are (b) (4)%, respectively. (b) (4) is also specified in the drug product, in which the proposed acceptance limit is (b) (4)%. The proposed acceptance limits for each of the impurities listed above are qualified by the cited toxicity studies in rats, and are therefore acceptable. Details of the qualification assessments are summarized in Table 107 and Table 108. The proposed maximum daily dose for maralixibat is 407 µg/kg/day (0.407 mg/kg/day), based on the weight-based dosing instructions. Therefore, safety assessment of the impurities is based on the assumed dose of 407 µg/kg/day maralixibat (i.e., 28.5 mg/day administered in patients weighing 70 kg, based on the dosing instructions).

Table 107. Qualification of Impurities (b) (4) and (b) (4) in Drug Substance and/or Drug Product

Parameter	Drug Substance Impurity (b) (4)	Drug Product Impurity (b) (4)	Drug Substance Impurity (b) (4)
Human			

Parameter	Drug Substance Impurity (b) (4)	Drug Product Impurity	Drug Substance Impurity
Rat			
Rat/human ratio			

Source: Prepared by the nonclinical reviewer

(b) (4)

Table 108. Qualification of Impurities (b) (4) and (b) (4) in Drug Substance

Parameter	(b) (4)
Human	
Rat	
Rat/human ratio	

Source: Prepared by the nonclinical reviewer.

(b) (4)

Justification for Specification of (b) (4) in Drug Substance

(b) (4) is considered a Class 5 impurity based on ICH M7(R1) and can be controlled as a nongenotoxic impurity. The proposed acceptance criterion in the drug substance is (b) (4)% area, which will allow for a maximum daily intake of (b) (4) mg at the recommended maximum daily dose of 28.5 mg maralixibat in human. Therefore, the proposed acceptance criterion is acceptable based on compliance with the recommendations in ICH Q3A(R2), and no qualification is needed.

Justification of Specification of (b) (4)

The impurity (b) (4) is a degradant in the drug product (Figure 17). It is considered a Class 5 impurity based on ICH M7(R1); therefore, it can be controlled as a nongenotoxic impurity. The proposed acceptance criterion in the drug product is (b) (4)% area, which will allow for a

maximum daily intake of (b) (4) mcg at the recommended maximum daily dose of 28.5 mg maralixibat. The proposed acceptance criterion is acceptable based on compliance with the qualification threshold stated in ICH Q3B(R2).

Figure 17. Structure of (b) (4)

(b) (4)

Source: Applicant's report 3.2.P.5.5 Characterization of Impurities.

Toxicological Assessment of Impurity (b) (4)

Due to concerns from the nonclinical and chemistry, manufacturing, and controls teams about impurity (b) (4) in the drug substance, the FDA sent the following Information Request on May 17, 2021:

"We refer to the drug substance impurity (b) (4), which is described in the report titled "3.2.S.3.2 Impurities." We do not agree with your assumption that this impurity is purged and removed during the manufacturing of the drug substance. Furthermore, you have not provided an analysis of (b) (4) in the drug substance. Based on your proposed drug substance specification, which does not include an acceptance criterion for (b) (4), we calculated that the maximum potential daily dose of (b) (4) from maralixibat will be (b) (4) mg (based on the maximum recommended dose of 28.5 mg/day). Therefore, you need to provide information to support the safety of (b) (4) in the drug substance, as follows:

*1. Although you stated that (b) (4) was negative in the Ames test (bacterial mutagenicity), you have not submitted the study report. We request that you submit the full report of the Ames test for (b) (4), to justify that (b) (4) can be controlled as a non-genotoxic i*mpurity.

2. Assuming that (b) (4) can be controlled as a non-genotoxic impurity based on the Ames test results, you will also need to provide safety information to qualify the exposure to (b) (4) at (b) (4) mg/day. Information that could be used to support qualification includes published toxicology studies, or the available toxicology studies with maralixibat that may have included sufficient levels of (b) (4) in the test article. If no information is available to support

qualification, you will need to specify [redacted] *at* [redacted]*% area, in accordance with ICH Q3A(R2).”*

The Applicant responded and provided a toxicological assessment of this impurity (amendment dated May 24, 2021). The key information submitted is reviewed below.

[redacted] was not mutagenic in the Ames assay. In a micronucleus assay, mice were treated with [redacted] at oral doses of [redacted] mg/kg for 1 day and a dose of [redacted] mg/kg for 3 days, followed by sacrifice for analysis of polychromatic erythrocytes. [redacted] was negative in the micronucleus assay in mice [redacted]

Since [redacted] should be controlled as a nongenotoxic impurity in the drug substance, the recommendations provided in [redacted] However, the maximum potential daily dose of [redacted] from maralixibat will be [redacted] µg ([redacted] µg/kg/day based on the maximum recommended dose of 28.5 mg/day administered in patients weighing 70 kg), thereby exceeding the qualification threshold.

The Applicant provided a summary of published toxicology studies with [redacted]. For safety assessment, the Applicant calculated the permitted daily exposure (PDE) [redacted]. This study and the PDE calculation are described below.

[redacted] was administered to male and female rats by oral gavage at doses of [redacted] mg/kg/day. Male animals were dosed for 28 days, beginning 14 days prior to mating and continuing throughout the 14-day mating period. Females were dosed for 57 days, beginning 14 days prior to mating and continuing until PND 4. In parental animals, [redacted] at [redacted] mg/kg/day produced decreases in body weight, food consumption, and motor activity (females), and increases in alkaline phosphatase concentrations (females) and liver weights (females). [redacted] also produced microscopic changes, including inflammatory and/or proliferative lesions in the kidneys (both sexes at [redacted] mg/kg/day) and urinary bladder (in a single female). None of these findings were observed after the 14-day recovery period.

Developmental toxicity (embryo-fetal and postnatal) was observed at [redacted] mg/kg/day. These effects included an increase in resorptions and decreases in live litter size, postnatal pup survival, and pup body weights. There were no external anomalies in the pups. The NOAEL for parental toxicity was considered to be [redacted] mg/kg/day (based on kidney lesions at [redacted] mg/kg/day). The NOAEL for developmental toxicity was considered to be [redacted] mg/kg/day [redacted]

Based on the NOAEL for parental animals, the calculated PDE for [redacted] is [redacted] µg/kg/day (details of calculation are shown below). Thus, the PDE is approximately [redacted] fold the maximum potential exposure to [redacted] from the recommended maximum daily dose of

maralixibat. Therefore, there is a reasonable assurance of safety for the presence of (b) (4) in maralixibat.

(b) (4)

14. Clinical Pharmacology: Additional Information and Assessment

14.1. In Vitro Studies

Maralixibat is minimally absorbed into systemic circulation following oral administration. In the clinical studies conducted in pediatric subjects with ALGS, plasma concentrations were below the LLOQ (0.25 ng/mL) after multiple doses at therapeutic dose levels (70 to 400 mcg/kg/day). The highest plasma concentration observed in the clinical studies in subjects with ALGS was 5.93 ng/mL in Study LUM001-304, which was used as the threshold for evaluation of in vitro data.

Protein Binding (Study M3099225)

The binding of maralixibat to human serum albumin and alpha-1-acid glycoprotein was determined by ultracentrifugation method at concentrations of 0.025, 0.25, 2.5 and 250 mcg/mL following incubation at 37°C for 1 h. The specific protein binding of maralixibat in human serum albumin was >91% and concentration-independent over the 0.025 to 25 mcg/mL range. The specific protein binding of maralixibat to alpha-1-acid glycoprotein was >93% and independent of concentration over the range of concentrations evaluated. The mean percentages of plasma protein binding were 95.9, 97.3, and 96% at concentrations of 0.25, 2.5 and 25 mcg/mL, respectively.

In Vitro Metabolism of Maralixibat (Study M3099002)

In vitro metabolism of maralixibat was studied in liver microsomes using [^{14}C] maralixibat (10 μg/mL). In pooled human liver microsomes, maralixibat was extensively metabolized with 69.4% of 10 μg/mL [^{14}C] maralixibat metabolized in a 60 min incubation period.

The metabolism of maralixibat occurred in vitro via two major routes: N-demethylation to form M1 and hydroxylation to form M3. The subsequent hydroxylation of M1 or N-demethylation of M3 yielded M4. The further N-demethylation of M1 generated M2. The subsequent

hydroxylation of M2 or N-demethylation of M4 formed M5. The further hydroxylation of M3 yielded M6. The Applicant has not conducted reaction phenotyping studies to identify the drug-metabolizing enzymes that are involved in the metabolism of maralixibat.

In Vitro Drug Interactions of Maralixibat (Studies 25582, XT165057, and XT163049)

Inhibition and Induction of Cytochrome P450 Enzymes

The inhibitory effects of maralixibat on the activities of various human recombinant cytochrome P450 (CYP) isoforms (i.e., 1A2, 2C9, 2C19, 2D6, and 3A4) were assessed at concentrations of 0.01 to 10μM. Of the various CYP isoforms evaluated, maralixibat showed inhibition of CYP3A4, (IC_{50} 3 to 10.9μM), with some evidence for time-dependent inhibition of CYP3A4. The potential for inactivation of CYP3A4 by maralixibat was further investigated by the Applicant and a propensity for time-dependent inactivation was observed (Kinact, 0.015 min^{-1}; Ki, 5.1μM). The Applicant used a population-based pharmacokinetics (PBPK) model to assess the clinical drug interaction potential via inhibition of CYP3A4 and concluded that significant inhibition of CYP3A4 by maralixibat is unlikely at the proposed therapeutic doses. Additional analysis conducted by the review team using the static model led to a similar conclusion. Refer to Section 14.4 (PBPK modeling) for details.

No significant induction was observed for CYP1A2, CYP2B6, or CYP3A4 at the concentrations evaluated.

Inhibition of Transporters

The interaction of maralixibat with human P-glycoprotein (MDR1), breast cancer resistance protein (BCRP), octamer-binding transcription factor 3, organic anion transporting polypeptide 1B1 (OATP1B1), OATP2B1, organic cation transporter 1 (OCTN1), OCTN2, multidrug resistance-associated protein 2 (MRP2), and peptide transporter 1 (PEPT1) was assessed at concentrations up to 10μM. Maralixibat is not a substrate of BCRP, MDR1, OATP1B1, OATP1B3, and OATP2B1.

Maralixibat inhibited BCRP, octamer-binding transcription factor 3, OATP1B1, OATP2B1, OCTN1, OCTN2, and MRP2-mediated transport of probe substrate by 13.1%, 16.3%, 96.6%, 95.3%, 27.3%, 50.2%, and 65.8%, respectively. Considering the low systemic absorption of maralixibat, only the interaction with OATP2B1 may be relevant due to its expression in the GI tract. The IC_{50} for inhibition of OATP2B1 by maralixibat was determined to be 1.02μM (Study OPT-2012-150). Given the high local concentrations of maralixibat in the GI tract after oral dosing (considering the highest proposed dose of 380 mcg/kg/day), inhibition of OATP2B1 in the GI tract is plausible (R >10).

In clinical studies, coadministration of 4.75 mg maralixibat (once daily) with daily doses of simvastatin, lovastatin, or atorvastatin did not have a clinically relevant effect on the pharmacokinetics of statins and their metabolites. However, the effect of maralixibat on the pharmacokinetics of OATP2B1 substrates at the highest proposed dose of 400 mcg/kg/day (380 mcg/kg/day free-base equivalent) has not been evaluated in a clinical study. Refer to Section 14.2 for more details.

14.2. In Vivo Studies

Clinical studies supporting clinical pharmacology information of maralixibat are summarized in Table 109.

Table 109. Summary of Clinical Studies Supporting Clinical Pharmacology Information of Maralixibat

Study	Study Objectives	Study Design	Subject Entered/ Completed; M/F; Age	Treatment
NB4-02-06-002	Single oral dose safety, tolerability, plasma and whole blood PK, and serum PD	Randomized, double-blind, placebo-controlled, ascending single oral doses, in healthy adult subjects	82/82; 71M/11F; 20-45 years	Single dose: Maralixibat 1, 2.5, 5, 10, 20, 50, 100, 300, and 500 mg before a meal; 10 mg fasted (n=6 per dose, 60 total); placebo (n=22)
NB4-02-06-003	Multiple oral dose safety, tolerability, PK, and serum and fecal PD, and efficacy	Randomized, double-blind, placebo-controlled, ascending multiple oral doses, in healthy adult subjects	167/163; 115M/52F; 21-63 years	QD for 28 days; maralixibat 0.5, 1, 2.5, 5,10, 20, 60, 100 mg (n=8 to 56 per dose, 147 total); placebo (n=20)
NB4-02-06-004	ADME study	Single-dose, open-label, in healthy male adult subjects	8/8; 8M/0F; 21-40 years	Single dose [^{14}C]maralixibat (5 mg, approximately 100 µCi) (n=8)
MRX-102	Food effect study	Single oral dose, single-blind, randomized, three-cohort in healthy adult subjects	36/36; 15M/21F; 18-55 years	30 mg (Fasted) 30 mg (Fed) 45 mg (Fasted) 45 mg (Fed) 100 mg (Fasted)
LUM001-301	Safety and efficacy PK collected	Double-blind, randomized, placebo-controlled, parallel group in pediatric patients 1 to 18 years of age with ALGS	37/35; 21M/16F; 1-17 years	Maralixibat 70, 140, and 280 µg/kg/day for 13 weeks (n=6-11 each dose, 25 total); placebo (n=12)
LUM001-302	Safety and efficacy PK collected	Double-blind, placebo-controlled, in pediatric patients 1 to 18 years of age with ALGS	20/19; 10M/10F; 1-16 years	Maralixibat 140 and 280 µg/kg/day for 13 weeks (n=6-8 each dose, 14 total); placebo (n=6)
LUM001- 303	Long-term safety and efficacy PK collected	Open-label in ALGS	19/6, 10M/9F; 1-16 years	Maralixibat 280 µg/kg/day QD, increased to BID
LUM001-304	Safety and efficacy PK collected	Randomized, placebo-controlled, drug-withdrawal with an open-label extension in children with ALGS	31 (entered)/ 29 (randomized); 19M/12F; 1-15 years	Maralixibat 400 µg/kg/day QD (Week 0-18: n=31; Week 19-22: n=13; Week 48 onward [could increase to 400 µg/kg BID] n=29)
LUM001-305	Long-term safety and efficacy PK collected	Open-label in ALGS	34/21, 20M/14F; 1-17 years	Maralixibat 280 µg/kg/day QD

Source: Adapted from Tables 9 and 16; Section 2.7.2., Summary of Clinical Pharmacology Studies.
Abbreviations: ADME, absorption, distribution, metabolism, excretion; ALGS, Alagille syndrome; BID twice daily; F, female; M, male; PD, pharmacodynamics; PK, pharmacokinetic; QD, once daily

Formulations

Phase 1 studies in healthy subjects were conducted with capsules or IR tablets. All the efficacy and safety studies in pediatric patients with ALGS (LUM001-301, 302, 303, 304, 305) were conducted with varying concentrations of maralixibat chloride (0.14 to 50 mg/mL) and fixed-dosing volumes. However, the to-be-marketed (TBM) commercial formulation has a similar composition to the fixed-dosing volume version, but with fixed drug substance concentrations and variable dosing volumes to achieve the desired weight-based dosing.

No in vivo comparative pharmacokinetic (PK) study was conducted between the TBM formulation and the previously used formulations as both formulations are oral solutions, and no excipients that significantly affect drug absorption (b) (4) were included. Refer to the biopharmaceutics review regarding the differences in the composition of the two formulations and any potential effects of the excipients on the oral bioavailability.

14.2.1. Phase 1 Single Ascending Dose Study (NB02-06-002)

Title

A **Randomized, Double-Blind, Placebo-Controlled, Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Study of Ascending Single Oral Doses of SD-5613 in Healthy Adult Subjects**

Objectives

The primary objective was to evaluate the safety and tolerability of single, oral doses of maralixibat in healthy adult subjects. The secondary objectives were to evaluate the (a) plasma, whole blood, and urine PK profiles of single oral doses of maralixibat administered to fed and fasted subjects and (b) sBA concentration and fBA excretion as a pharmacodynamic (PD) marker, prior to and following dosing with maralixibat or placebo.

Study Design

This was a randomized, double-blind, placebo-controlled study of ascending single oral doses of maralixibat. Subjects received single doses of maralixibat (1, 2.5, 5, 10, 20, 50, 100, 300, and 500 mg) or matching placebo immediately prior to consuming a morning meal after having fasted overnight (minimum of 12 h). There was one dosing panel in which subjects received a single dose of 10 mg maralixibat or matching placebo while fasting for 5 h post-dosing. The purpose of this panel was to evaluate PD and PK responses in the fasted state.

Eighty-two (82) subjects were randomized in this study: 60 subjects received maralixibat and 22 received placebo. Subjects were placed on a study diet with a fixed high-fat composition and a fixed caloric content to amplify the postprandial rise and reduce the variability of serum total bile acids. Plasma, whole blood, and urine PK samples were collected up to 96 h postdose.

PK Results

Concentrations were below the LLOQ (0.25 ng/mL) in all subjects at doses <20 mg when the drug was administered immediately before breakfast. PK parameters could only be estimated reliably at doses of 100 mg or higher. In the 500 mg dose group, the mean C_{max} was 2.40 ng/mL, the AUC_{0-24h} was 14.19 ng•h/mL, the T_{max} was 2.8 h, and the mean elimination half-life ($t_{1/2}$) was 3.8 h. The increases in AUC_{0-24h} and mean C_{max} were dose related in the 20 to 500 mg maralixibat groups. See Table 110.

Table 110. Pharmacokinetic Parameters of Plasma Maralixibat (Single Ascending Dose)

Dose (mg)	n	Mean (SD) PK Parameters[1]					
		C_{max} (ng/mL)	AUC_{0-24} (ng•h/mL)	AUC_{0-96} (ng•h/mL)	AUC_{0-inf} (ng•h/mL)	T_{max} (h)	$t_{1/2}$ (h)
1	6	0.000	0.000	0.000	NA	NA	NA
2.5	6	0.000	0.000	0.000	NA	NA	NA
5	6	0.000	0.000	0.000	NA	NA	NA
10	6	0.000	0.000	0.000	NA	NA	NA
10F[2]	6	0.454 (0.1594)	0.946 (0.4307)	3.27 (4.2368)	NA	2.000 (1.500, 4.000)	NA
20	6	0.081 (0.1972)	0.161 (0.3944)	0.161 (0.3944)	NA	6.000 (6.000, 6.000)[5]	NA
50	6	0.310 (0.4133)	0.600 (0.8516)	0.600 (0.8516)	NA	2.000 (1.500, 4.000)[6]	NA
100	6	0.727 (0.3854)	1.668 (0.6418)	1.668 (0.6418)	NA	2.500 (1.000, 4.000)	NA
300	6	2.078 (0.3296)	7.51 (1.9811)	7.510 (1.9811)	7.798 (3.6340)[3]	3.000 (1.500, 3.000)	2.023 (0.4701)[3]
500	6	2.401 (0.3441)	14.191 (7.5991)	14.812 (9.0517)	16.827 (11.7902)[4]	2.500 (2.000, 4.000)	3.791 (3.3742)[4]

Abbreviations: AUC = area under concentration-time curve; AUC_{0-24} = AUC from time 0 to 24 hours postdose; AUC_{0-96} = AUC from time 0 to 96 hours postdose; AUC_{0-inf} = AUC from time 0 to extrapolated infinity; C_{max} = maximum observed concentration; N = number of observations; NA = not available; PK = pharmacokinetics; SD = standard deviation; $t_{1/2}$ = elimination half- life; T_{max} = time of maximum observed concentration

1 PK parameters are presented as mean (SD), except for T_{max} that is presented as median (minimum, maximum).

2 Dose was administered under the fasted condition.

3 n = 2

4 n = 5

5 n = 1

6 n = 3

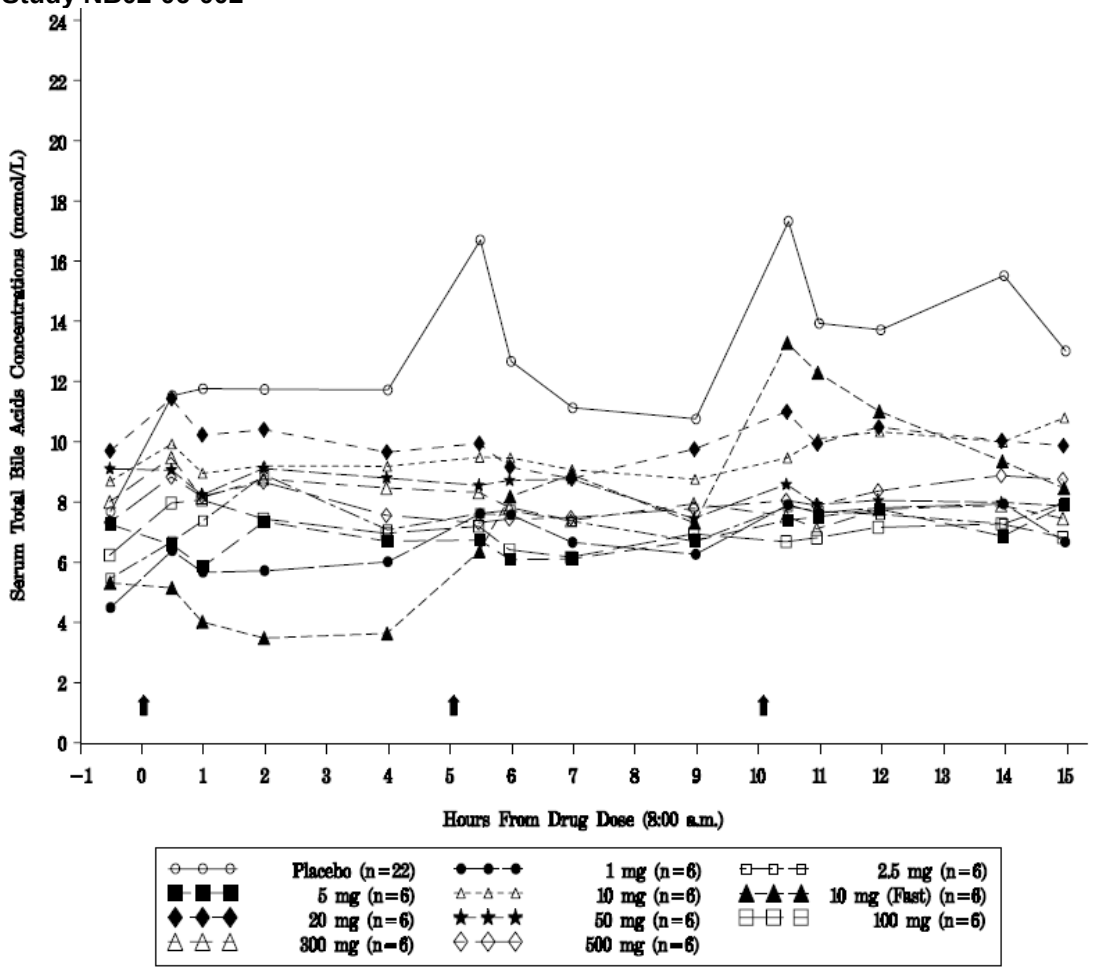
Source: Clinical study report for NB02-06-002.

PD Results

Total Serum Bile Acids

The normal postprandial increases in sBA concentrations were inhibited on Day 1 after administration of single doses of maralixibat in the range of 1 to 500 mg (Figure 18). The inhibition of the postprandial increases in sBA concentrations by maralixibat was rapid (within 30 min of meal consumption and within 60 min of oral dosing). The duration of inhibition of the postprandial increases in sBA concentration by maralixibat was prolonged at all doses, except the fasting 10 mg group. The inhibition was observed over the entire 15 h sampling period on Day 1 and was also observed for at least the initial 4 h of Day 2.

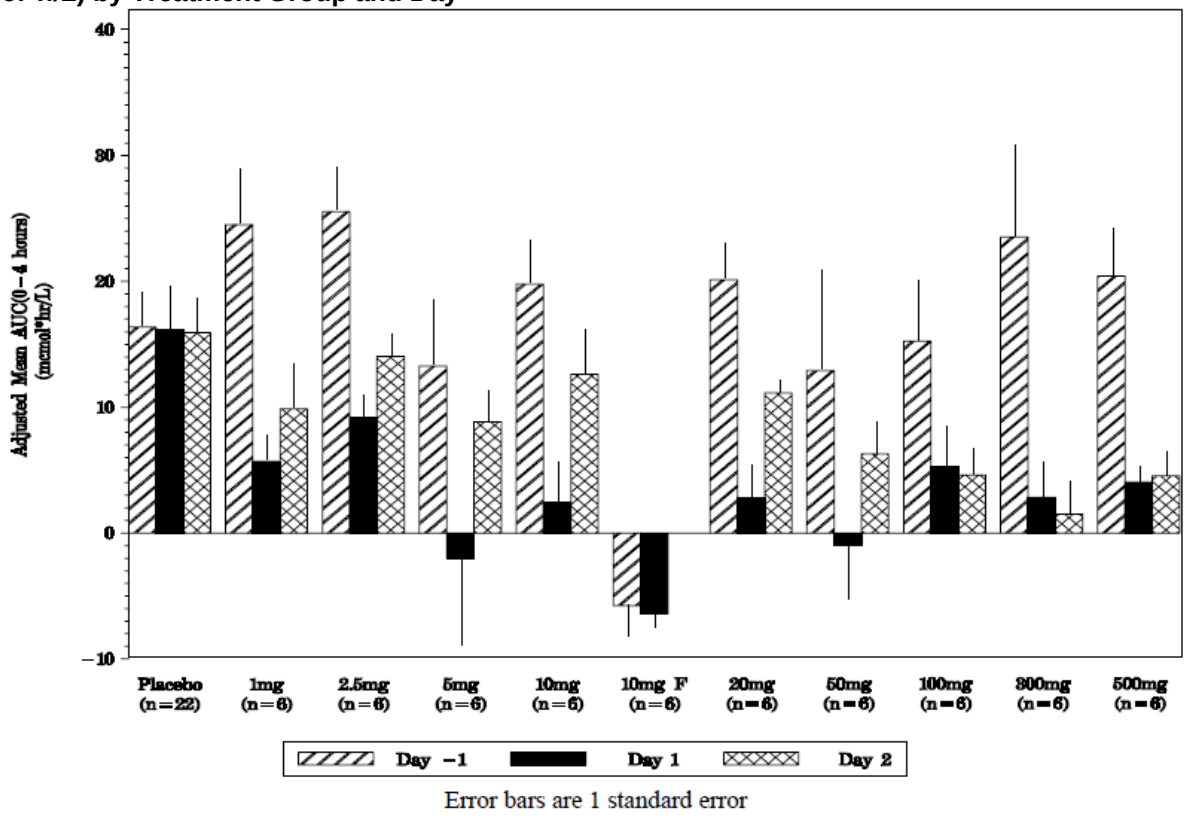
Figure 18. Mean Serum Total Bile Acids Concentration on Day 1 by Treatment Group and Time, Study NB02-06-002



Source: Figure 8b from the clinical study report for NB02-06-002.
Arrows indicate meal intake.

Reductions in mean sBA AUC (baseline adjusted and unadjusted) over 0 to 4 h on Day 1 approached saturation in the 1 mg dose group (the lowest dose tested) in that similar reductions in higher dose groups were observed over this period (Figure 19). There was a modest dose-dependent reduction in mean baseline adjusted sBA AUC over 0 to 15 h on Day 1 because the 1 and 2.5 mg doses caused less sBA AUC reduction than the 5 to 500 mg doses. There was a trend for dose dependence of the sBA AUC reduction on Day 2 because doses ≥50 mg demonstrated a greater reduction in mean baseline adjusted sBA AUC from time 0 to 4 h postdose and sBA AUC from time 0 to 4 h postdose compared to the lower-dose groups. In subjects who received 10 mg maralixibat followed by a 5 h fast, inhibition of the postprandial increase of sBA concentrations preceding lunch or dinner was not observed.

Figure 19. Baseline Adjusted Serum Total Bile Acids Concentration: Mean AUC (0 to 4 h; µmol•h/L) by Treatment Group and Day



Error bars are 1 standard error

Note: One standard error is displayed on the chart.
Note: Baseline adjusted AUC is Area Under the Curve above (that day's) Baseline.
Note: 10 mg F is the fasting 10 mg group.
Note: On Day 1 at Hour 2, subject (b) (6) (10 mg F group), had a sample identified as 'Below Detectable Limit.' For calculation of serum bile acid AUCs, this value was set to 0.00.
Note: As per protocol, no serum total bile acid samples were collected for the fasting 10 mg panel on Day 2.

Source: Figure 8d from the clinical study report for NB02-06-002.

Total Fecal Bile Acid Excretion

Mean fBA excretion increased in all maralixibat-treated groups on Day 1, consistent with decreases in mean sBA concentrations. On Day -1, mean fBA excretion was roughly similar among the 11 groups, ranging from 110 to 453 µmol/day. On Day 1, increases in mean fBA excretion were observed in all maralixibat-treated groups compared to placebo and ranged from 354 to 2440 µmol/day. There was no apparent dose-response relationship on Day 1. On Day 2, the mean fBA returned to Day -1 levels for most groups and ranged from 6.7 to 564.4 µmol/day.

14.2.2. Phase 1 Multiple Ascending Dose Study (NB4-02-06-003)

Title

A Randomized, Double-Blind, Placebo-Controlled, Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Study of Ascending Multiple Oral Doses of SD-5613 in Healthy Adult Subjects

Objectives

The primary objective of this multiple-dose study was to evaluate the safety and tolerability of escalating doses of oral maralixibat, administered once daily in the morning over a 28-day dosing interval, in healthy adult subjects. The secondary objectives were (a) to analyze the PK profile of maralixibat; (b) to determine the PD properties of maralixibat using serum and fecal total bile acids as biomarkers; (c) to evaluate the efficacy of various maralixibat doses using low-density lipoprotein cholesterol and other lipid parameters; and (d) to evaluate the effect of maralixibat on parameters of fat absorption.

Study Design

This was a randomized, double-blind, placebo-controlled study of multiple oral doses of maralixibat. Subjects were otherwise-healthy individuals with borderline and mildly elevated low-density lipoprotein cholesterol levels (130 to 190 mg/dL; 3.36 to 4.91 mmol/L) and with triglycerides ≤300 mg/dL (7.76 mmol/L). There were 13 dosing cohorts in which subjects received maralixibat once daily for 28 days: 0.5 mg every morning (qAM), 1 mg qAM, 2.5 mg qAM, 2.5 mg qAM repeat, 5 mg qAM, 5 mg qAM repeat, 5 mg every evening (qPM), 0.5 to 5 mg qAM dose escalation, 10 mg qAM, 20 mg qAM, 20 mg qAM repeat, 60 mg qAM, 100 mg qAM. The 10 to 100 mg qAM dosing panels included subjects treated with matching placebo. A total of 147 subjects was treated with maralixibat, and 20 subjects with placebo. Subjects were placed on a diet of 35% fat composition, fixed FSV composition and fixed caloric content to reduce the variability of serum and fecal total bile acids, lipid measurements, and fat absorption parameters. Plasma PK samples were collected at predose and up to 24 h postdose following dosing on Days 1 and 14.

PK Results

Following once daily multiple doses, maralixibat concentrations were generally not detectable (LLOQ 0.25 ng/mL) at doses ≤20 mg. Plasma PK parameters could only be calculated for the 60 and 100 mg dose groups. For the 100 mg dose on Day 14, mean C_{max} was 1.146 ng/mL, median T_{max} was 2.5 h, AUC_{last} was 4.6 ng•h/mL, and mean half-life ($t_{1/2}$) was 4.3 h (Table 111). There is no clear evidence for increased absorption or systemic accumulation of maralixibat after multiple doses.

Table 111. Pharmacokinetic Parameters of Plasma Maralixibat in the MAD Study (NB4-02-06-003)

Dose (mg)	n	Mean (SD) PK Parameters[1]			
		C_{max} (ng/mL)	AUC_{0-24} (ng•h/mL)	T_{max} (h)	$t_{1/2}$ (h)
0.5	16	0.000	0.000	NA	NA
1	8	0.000	0.000	NA	NA
2.5 (qAM)	8	0.000	0.000	NA	NA
5	8	0.032 (0.0910)	0.032 (0.0910)	3.000 (3.000, 3.000)[2]	NA
10	8	0.129 (0.1852)	0.248 (0.4170)	2.000 (1.000, 4.000)[3]	NA
20 (qAM)	8	0.376 (0.1902)	1.152 (0.7792)	3.000 (0.500, 4.000)[4]	1.275 (NA)[2]
20 (qAM Repeat)	8	0.310 (0.3421)	0.424 (0.4083)	1.000 (0.500, 3.000)[5]	NA
60	8	0.781 (0.256)	5.000 (2.2034)	3.000 (0.500, 23.500)	4.367 (0.2818)[6]
100	8	1.146 (0.3267)	4.614 (1.7994)	2.500 (0.500, 4.000)	4.282 (1.1139)[6]

Abbreviations: AUC = area under concentration-time curve; AUC_{0-24} = AUC from time 0 to 24 hours postdose; C_{max} = maximum observed concentration; N = number of subjects; NA = not available; PK = pharmacokinetics; qAM = every morning; SD = standard deviation; $t_{1/2}$ = elimination half- life; T_{max} = time of maximum observed concentration.

1 PK parameters are presented as mean (SD), except for T_{max}, which is presented as median (minimum, maximum).

2 n = 1

3 n = 3

4 n = 7

5 n = 5

6 n = 2

Source: Table 11 in the Summary of Clinical Pharmacology.

PD Results

Total Serum Bile Acids

At dose levels of 0.5 to 100 mg, inhibition of postprandial increases of sBA concentrations was observed; however, the inhibition was attenuated at Day 14 compared with Day 1, particularly at 9 to 15 h postdose.

Total Fecal Bile Acid Excretion

Over Days 9 to 14, doses of maralixibat >5 mg led to increases in excretion of fBAs compared with placebo; over Days 23 to 28, increases were observed across the entire 1 to 100 mg dose range evaluated (Table 112).

Table 112. Summary of Daily Total Fecal Bile Acids Excretion in the Multiple Ascending Dose Study (NB4-02-06-003)

Dose (mg) or Placebo	N	Mean (SD) Daily Total Fecal Bile Acids Excretion (µmol)	
		Days 9 to 14	Days 23 to 28
Placebo	16	154.58 (161.50)	163.39 (182.13)
0.5	16	266.84 (209.91)	294.94 (173.02)
1.0	8	642.70 (439.36)	780.29 (670.54)
2.5 (qAM)	8	477.95 (403.09)	590.71 (281.49)
5 (qAM)	8	1105.08 (863.17)	848.37 (683.95)
5 (qPM)	16	514.33 (340.23)	593.25 (437.66)
10	8	1236.96 (685.04)	1126.04 (434.47)
20 (qAM)	8	1140.28 (540.62)	1030.58 (370.71)
20 (qAM)	8	665.54 (468.30)	699.77 (511.10)
60	8	973.39 (759.29)	964.54 (683.51)
100	8	2405.71 (843.08)	1718.27 (889.20)

Abbreviations: N = number of subjects; SD = standard deviation; qAM = every morning; qPM = every evening.

Note: The total excretion over the 6-day period is divided by 6 for each subject prior to the calculation of summary statistics.

Note: Subjects who did not produce a sample within a 24-hour collection period have an assigned excretion value of zero and are included in the data.

Source Clinical study report for NB4-02-06-003.

14.2.3. Mass Balance Study (NB4-02-06-004)

Title

A Pharmacokinetic Study of Single Oral Doses of [14C]SD-5613 (Maralixibat) in Healthy Male Subjects

Objective

To determine the absorption, distribution, metabolism, excretion profile of an oral solution of [^{14}C]maralixibat.

Study Design

This was an open-label, one-period, single-dose study of a [^{14}C]maralixibat oral solution. Eight healthy male subjects received an oral solution of 5 mg [^{14}C]maralixibat (containing approximately 100 µCi per dose). Blood samples were collected at predose and for 72 h postdose, urine samples were collected predose and for 168 h postdose, and feces were collected predose and for 216 h postdose. Plasma, whole blood, urine, and fecal samples were analyzed for total radioactivity, and selected fecal samples were analyzed for maralixibat and relevant metabolite concentrations. In addition, metabolic profiles in selected fecal samples were obtained using high-performance liquid radiochromatography.

Results

Less than 1% of the radioactive dose was detected in plasma, whole blood, or urine. Total radioactivity recovery was 72.532%, with 72.466% found in the feces and 0.066% in the urine. Radioactivity detected in the plasma and whole blood was below the LLOQ. Since levels of radioactivity in the plasma, blood, and urine were low or undetectable, the metabolic profile of

maralixibat in these matrices could not be determined. Fecal profiling showed that radioactivity was predominantly present as the parent compound (>94%). Three metabolites were identified in the feces in relatively low amounts. These were N-demethylated maralixibat (range, ≤1% to 2.26%), mono-hydroxylated maralixibat (hydroxylation in the butyl side chain; range, 1.08% to 5.54%) and mono-demethylated, mono-hydroxylated maralixibat (range, ≤1% to 1.62%). These data suggest minimal maralixibat systemic absorption and that most of the administered maralixibat was excreted as unabsorbed parent drug in the feces. Minor metabolites were detected in the feces; however, it is unclear if these were formed following absorption or in the gut.

14.2.4. Food Effect Study (Study MRX-102)

Title

A Phase 1 Single-Blind, Randomized Study to Assess the Single-Dose Pharmacokinetics of a To-Be-Marketed Liquid Formulation of Maralixibat at Different Dose Levels and Fasting Conditions

Objectives

- To evaluate the effect of food on the PK of single doses of 30 or 45 mg maralixibat administered as the TBM liquid formulation.
- To evaluate the PK of maralixibat after a single dose of 100 mg of the TBM liquid formulation.
- To collect replicate 12-lead electrocardiograms from continuous electrocardiogram Holter monitoring data after fasted administration of maralixibat or placebo for possible analysis.
- To assess the safety and tolerability of single oral doses of 30, 45 or 100 mg of the TBM liquid formulation of maralixibat in healthy participants.

Study Design

This was a single-center, single-blind, randomized, three-cohort study in healthy adult participants. Twelve participants were enrolled in each of three cohorts for a total of 36 participants. The first 12 eligible participants were enrolled in Cohort 1 (single doses of 30 mg maralixibat randomized to the fed-fasted or fasted-fed states). The second group of 12 eligible participants who signed informed consent forms was assigned to Cohort 2 (single doses of 45 mg maralixibat randomized to the fed-fasted or fasted-fed states). A standard high-fat breakfast was used as the fed condition. The third group of 12 eligible participants was enrolled in Cohort 3 (100 mg maralixibat administered in the fasted state). Cohort 3 provided time-matched electrocardiogram and plasma concentration data at a supratherapeutic dose for corrected QT analyses. Participants enrolled in Cohorts 1 and 2 completed two study periods separated by a washout period of at least 1 week. Cohorts 1 and 2 were randomized to receive their assigned maralixibat dose in one of two sequences: fasted followed by fed or fed followed by fasted. The total volume of solution was 240 mL for all treatments. PK blood samples were collected following each dose at predose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h postdose.

PK Results: Effect of Food

Following a single oral administration of maralixibat 30 and 45 mg liquid formulation under fed conditions, geometric mean plasma AUC_{last} and C_{max} achieved 22.9% to 45.6% of the values under fasted conditions (Table 113, Figure 20). Maralixibat median plasma T_{max} was delayed by approximately 2 to 2.5 h when administered with food compared to fasting conditions with median T_{max} values of 2.5 and 3 h under fed conditions versus 0.75 and 0.5 h under fasted conditions at the 30 and 45 mg doses, respectively. Table 114 summarizes the statistical analysis of AUC_{last} and C_{max} values. The relative BA of maralixibat following a single oral administration of maralixibat 30 or 45 mg under fed conditions was 64.8% to 85.8% lower than that of maralixibat following administration under fasted conditions. However, since maralixibat exerts its pharmacological effect by its local action in the small intestine, differences in the systemic PK of maralixibat in fasted versus states may not have a clinical impact from an efficacy standpoint. Of note, when maralixibat was administered under fasting conditions, the postprandial sBA increase was not inhibited in healthy subjects (Section 14.2.1); therefore, it is plausible that the increased systemic absorption affects the local concentration in the gut.

Table 113. Summary of Maralixibat Pharmacokinetic Parameters Following Administration of a Single Oral Dose of Maralixibat Under Fasted and Fed States

Plasma PK Parameters[a]	Maralixibat 30 mg Fasted (N=12)	Maralixibat 30 mg Fed (N=8)	Maralixibat 45 mg Fasted (N=12)	Maralixibat 45 mg Fed (N=9)	Maralixibat 100 mg Fasted (N=12)
AUC_{∞} (h*ng/mL)	5.74 (30.1); 4	NC	5.73 (35.6); 4	NC	13.0 (73.2); 10
AUC_{∞}/Dose (h*ng/mL/mg)	0.191 (30.1); 4	NC	0.127 (35.6); 4	NC	0.130 (73.2); 10
AUC_{last} (h*ng/mL)	2.24 (214.1); 12	0.514 (164.8); 8	3.07 (162.5); 12	1.40 (98.0); 9	10.2 (82.9); 12
AUC_{last}/Dose (h*ng/mL/mg)	0.0746 (214.1); 12	0.0171 (164.8); 8	0.0682 (162.5); 12	0.0310 (98.0); 9	0.102 (82.9); 12
C_{max} (ng/mL)	1.27 (99.0); 12	0.437 (44.5); 8	1.50 (55.2); 12	0.564 (45.7); 9	3.05 (52.3); 12
C_{max}/Dose (ng/mL/mg)	0.0423 (99.0); 12	0.0146 (44.5); 8	0.0334 (55.2); 12	0.0125 (45.7); 9	0.0305 (52.3); 12
T_{max}[b] (h)	0.75 (0.50 – 2.00); 12	2.50 (0.50 – 6.00); 8	0.50 (0.50 - 2.58); 12	3.00 (0.50 – 4.00); 9	0.75 (0.50 – 3.00); 12
$t_{1/2}$ (h)	1.61 (76.3); 4	NC	1.44 (66); 4	NC	1.97 (56.4); 10
T_{last}[b] (h)	4.00 (1.50 - 6.03); 12	4.00 (0.50 – 6.00); 8	5.00 (1.00 – 8.00); 12	4.00 (3.00 – 24.00); 9	8.00 (3.00 – 12.00); 12
CL/F (L/h)	5230 (30.1); 4	NC	7860 (35.6); 4	NC	7710 (73.2); 10
V_z/F (L)	12100 (46.3); 4	NC	16300 (30); 4	NC	22000 (56.7); 10

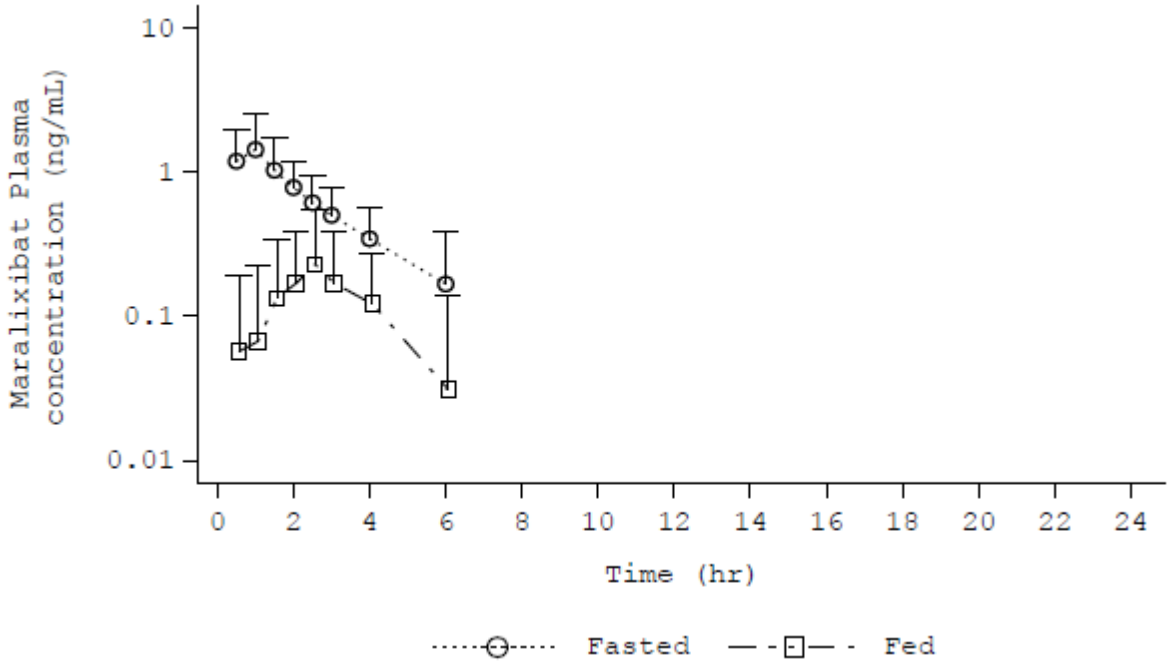
AUC=area under the concentration-time curve; AUC_{∞}=AUC from time 0 extrapolated to infinity; AUC_{last}=AUC from time zero to last measured timepoint; CL/F=apparent oral clearance; C_{max}=maximum concentration; NC=not calculated; PK=pharmacokinetic; $t_{1/2}$=half-life; T_{last}=time of the last point with quantifiable concentration; T_{max}=time to the maximum concentration; V_z/F=apparent volume of distribution during terminal phase.
[a]Geometric Mean (CV% GeoMean); n.
[b]Median (Min - Max); n.
Source: Clinical study report for Study MRX-102.

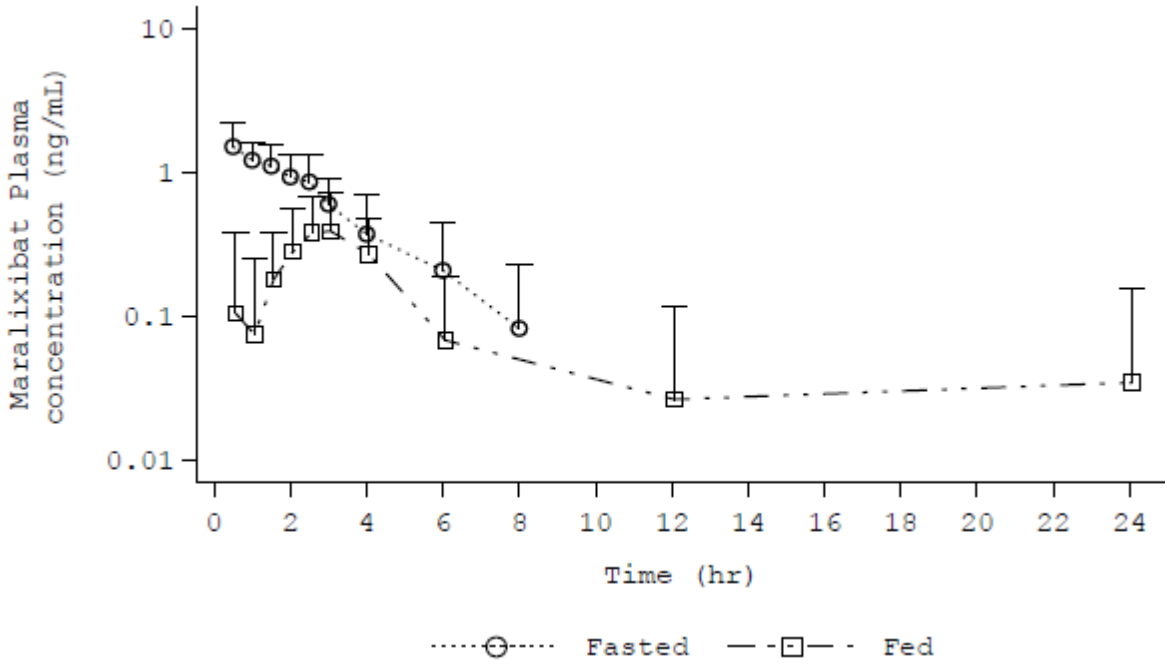
Table 114. Statistical Comparison of Maralixibat Pharmacokinetic Parameters in Fasted and Fed States Following Oral Administration

Cohort	PK Parameter	N	Geometric LSM (Fed)	Geometric LSM (Fasted)	Fed/Fasted Ratio (%)	90% CI for Geometric LS Mean Fed/Fasted Ratio
Maralixibat 30 mg	AUC_{last} (ng•h/mL)	8	0.51	3.60	14.2	5.80, 34.69
	C_{max} (ng/mL)	8	0.45	1.69	26.8	20.22, 35.56
Maralixibat 45 mg	AUC_{last} (ng•h/mL)	9	1.39	4.53	30.7	18.16, 51.92
	C_{max} (ng/mL)	9	0.57	1.62	35.2	27.80, 44.64

Abbreviations: AUC = area under concentration-time curve; AUC_{∞} = AUC from time 0 to extrapolated infinity; AUC_{last} = AUC from time 0 to last measurable concentration; CI = confidence interval; C_{max} = maximum observed concentration; LSM = least squares means; N = number of subjects; PK = pharmacokinetics;
Source: Clinical study report for Study MRX-102.

Figure 20. Mean Plasma Maralixibat Concentration Versus Time Profiles Following Oral Administration of 30 mg and 45 mg Maralixibat in Fasted Versus Fed States





Source: Clinical study report for Study MRX-102, Figures 11.1 and 11.2.

PK Results: Dose Proportionality

Following a single oral administration of maralixibat 30, 45, and 100 mg liquid formulations under fasted conditions, geometric mean plasma AUC_{last} and C_{max} increased in a dose-proportional manner by 4.6- and 2.4-fold, respectively, following a 3.3-fold dose increase from 30 to 100 mg. Median T_{max} was similar across the three dose levels, ranging from 0.5 to 0.75 h. Half-life was comparable across the three dose levels, ranging from 1.44 to 1.97 h. Statistical analysis of dose proportionality for the PK evaluable population is summarized in Table 115. Statistical analysis using the power model suggested that the PK of maralixibat (AUC_{last} and C_{max}) are dose proportional over the 30 to 100 mg dose level range since the 90% CI for the slope of the regression line contains 1.

Table 115. Statistical Comparison of Maralixibat Pharmacokinetic Parameters in Fasted and Fed States Following Oral Administration

PK Parameter (Units)	No. of Participants			Slope Estimate	90% CI	Intercept Estimate	p-value
	30 mg	45 mg	100 mg				
C_{max} (ng/mL)	12	12	12	0.75	0.40 - 1.10	-2.36	0.0010
AUC_{last} (h*ng/mL)	12	12	12	1.29	0.69 - 1.90	-3.67	0.0010

AUC_{last}=AUC from time zero to last measured timepoint; CI=confidence interval; C_{max}=maximum concentration; PK=pharmacokinetic.

Notes: All participants were in the fasted state at the time of assessment. Slope estimates and 90% CIs were obtained from a power model with log-transformed PK parameter as the dependent variable and log-transformed dose as the independent variable. The p-value estimate was whether the slope of the doses was significantly different from 0.

Source: Clinical study report for Study MRX-102

14.2.5. Clinical Drug-Drug Interaction Studies (NB4-02-06-008, NB4-01-06-019, NB4-02-06-020, LUM001-201)

Since maralixibat is minimally absorbed, the potential for systemic drug interactions with other drugs is unlikely at the recommended doses. However, drug interactions in the GI tract are plausible. The Applicant conducted three clinical drug-drug interaction studies investigating the effects of maralixibat administration on the PK and/or PD of lovastatin, simvastatin, and atorvastatin (substrates of OATP2B1 and CYP3A4) (Study NB4-02-06-008 in hypercholesterolemic subjects and Studies NB4-01-06-019 and NB4-02-06-020 in healthy subjects) See Table 116.

Table 116. Summary of Relevant Clinical Drug-Drug Interaction Pharmacokinetic Studies

Study	Study Objective	Study Design	Subject Entered/ Completed; M/F; Age	Treatments		
				Substrate	Interacting Drug	N
NB4-02-06-008	Efficacy and safety of maralixibat in coadministration with lovastatin or simvastatin	Randomized, double-blind, placebo-controlled, parallel group, in healthy adult subjects with high LDL	80/73; 51M/29F; 18-69 years	Lovastatin 20 mg QD for 28 days	Maralixibat 2.5 mg QD	20
					Placebo QD	20
				Simvastatin 20 mg QD for 28 days	Maralixibat 2.5 mg QD	21
					Placebo QD	19
NB4-01-06-019	DDI of maralixibat in coadministration with lovastatin or simvastatin	Open-label, randomized, 4-treatment, 2-sequence crossover, in healthy adult subjects	24/24; 8M/16F; 18-49 years	Lovastatin 20 mg QD for 5 days	Maralixibat 5 mg QD	24
					NA	24
				Simvastatin 20 mg QD for 5 days	Maralixibat 5 mg QD	24
					NA	24
NB4-02-06-020	Steady-state DDI between atorvastatin and multiple doses of maralixibat	Open-label, randomized, 4-treatment, 4-period, 2-sequence crossover, in healthy adult subjects	24/20; 14M/10F; 19-47 years	Atorvastatin 20 mg qAM for 5 days (N=20 or 23)	Maralixibat 5 mg qAM for 5 days	20
					NA	23
				Atorvastatin 20 mg qPM for 5 days (N=20 or 21)	Maralixibat 5 mg qAM for 5 days	20
					NA	23

Source: Reviewer generated table
Abbreviations: DDI, drug-drug interaction; F, female; LDL, low-density lipoprotein; M, male; PK, pharmacokinetics; qAM, every morning; qPM, every evening; QD, once daily

14.2.5.1. Study NB4-02-06-008

Title

A Randomized, Double-Blind Study Comparing SD-5613(Maralixibat)/Statin Combination Therapy and Statin Monotherapy in Healthy, Adult Subjects

Objectives

- The primary objective was to determine the efficacy and safety of maralixibat in co-administration with lovastatin or simvastatin in healthy, adult subjects.
- The secondary objectives were to evaluate the (a) safety of maralixibat combined with statin therapy, (b) effect of combination therapy on the PK profiles of maralixibat and statins, and (c) statin class effect of maralixibat/statin combination therapy.

Study Design

This was a single center, double-blind, randomized, parallel-group, placebo-controlled comparative study designed to assess a possible additive effect between maralixibat and a statin (lovastatin or simvastatin) with respect to lowering low-density lipoprotein-cholesterol over a 4-week treatment period in healthy, adult subjects.

There were four treatment groups in which subjects received maralixibat 2.5 mg qAM + lovastatin 20 mg qPM, maralixibat placebo qAM + lovastatin 20 mg qPM, maralixibat 2.5 mg qAM + simvastatin 20 mg qPM, or maralixibat placebo qAM + simvastatin 20 mg qPM.

Blood samples were collected for the determination of statin and metabolite plasma concentrations prior to dosing of statin (predose; 0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after dosing.

Maralixibat or maralixibat placebo was administered orally each day of the 28-day treatment period at approximately 0800, immediately prior to the morning meal, Statin treatment was administered orally each day of the 28-day treatment period at approximately 1800, immediately prior to the evening meal. The dosing timing for simvastatin and lovastatin is consistent with the recommended dosing in the evening per the labeling.

PK Results

Maralixibat plasma concentrations were below the assay sensitivity limit at nearly all timepoints evaluated in subjects who received maralixibat. After staggered dosing of statins from maralixibat, maralixibat did not result in altered PK parameters of lovastatin or its active metabolite, beta-hydroxylovastatin (Table 112). Staggered dosing of simvastatin from maralixibat resulted in 40 to 50% lower mean AUC and C_{max} of simvastatin compared to simvastatin alone. However, the PK parameters of the active metabolite, beta-hydroxysimvastatin, were generally unaffected.

Although literature reports support the role of OATP2B1 in the absorption of statins, the clinical implication of the change in simvastatin exposures with the proposed dosing regimen is unclear.

The dose of maralixibat (5 mg) used in this study was five-fold lower than the highest dose (400 mcg/kg/day, equivalent to ~28 mg for a 70 kg adult). In addition, the study design was not

designed to evaluate the worst effect on PK of OATP2B1 substrates (i.e., simultaneous administration of statins) because statins were dosed 10 h after maralixibat per the recommended dosing time for both drugs.

Table 117. Lovastatin, β-Hydroxylovastatin, Simvastatin, and β-Hydroxysimvastatin PK Parameters in the Presence of Maralixibat Versus Placebo

Victim Drug	Analyte	Day	PK Parameter	Geometric LS Mean Test (N=20)	Geometric LS Mean Reference (N=20)	Ratio of Test/Reference	90% CI for Ratio of Means
Lova	Lova	1	AUC_{last} (ng•h/mL)	11.86	14.21	0.834	0.598, 1.164
			$AUC_{0\text{-}inf}$ (ng•h/mL)	14.801	16.592	0.892	0.632, 1.260
			C_{max} (ng/mL)	2.74	3.90	0.701	0.485, 1.013
			T_{max} (h)	1.61	1.17	NA	NA
		14	$AUC_{0\text{-}24}$ (ng•h/mL)	12.522	16.49	0.759	0.554, 1.041
			C_{max} (ng/mL)	3.222	3.46	0.933	0.643, 1.353
			T_{max} (h)	1.642	1.71	NA	NA
	β-hlova	1	AUC_{last} (ng•h/mL)	22.74	17.76	1.280	0.950, 1.726
			$AUC_{0\text{-}inf}$ (ng•h/mL)	25.283	18.074	1.399	1.044, 1.873
			C_{max} (ng/mL)	2.61	2.54	1.028	0.744, 1.422
			T_{max} (h)	5.79	3.56	NA	NA
		14	$AUC_{0\text{-}24}$ (ng•h/mL)	24.092	24.62	0.978	0.723, 1.324
			C_{max} (ng/mL)	3.272	3.17	1.031	0.744, 1.431
			T_{max} (h)	3.602	4.01	NA	NA
Simva	Simva	1	AUC_{last} (ng•h/mL)	7.02	12.25	0.573	0.415, 0.792
			$AUC_{0\text{-}inf}$ (ng•h/mL)	8.551	14.03	0.610	0.444, 0.837
			C_{max} (ng/mL)	3.25	6.32	0.515	0.375, 0.707
			T_{max} (h)	1.60	0.85	NA	NA
		14	$AUC_{0\text{-}24}$ (ng•h/mL)	8.521	15.421	0.552	0.374, 0.816
			C_{max} (ng/mL)	4.161	7.371	0.565	0.369, 0.867
			T_{max} (h)	1.611	0.801	NA	NA
	β-hsimva	1	AUC_{last} (ng•h/mL)	9.34	7.87	1.187	0.810, 1.740
			$AUC_{0\text{-}inf}$ (ng•h/mL)	14.133	9.521	1.485	1.081, 2.041
			C_{max} (ng/mL)	1.04	0.86	1.218	0.878, 1.690
			T_{max} (h)	6.05	4.70	NA	NA
		14	$AUC_{0\text{-}24}$ (ng•h/mL)	7.931	9.671	0.820	0.534, 1.259
			C_{max} (ng/mL)	0.931	1.101	0.843	0.585, 1.215
			T_{max} (h)	4.301	3.891	NA	NA

Source: Clinical study report for NB4-02-06-008.
[1] n=18; [2] n=19; [3] n=16; [4] n=17
Abbreviations: β-hlova, β-hydroxylovastatin; β-hsimv, β-hydroxysimvastatin; AUC, area under the plasma-concentration time curve; $AUC_{0\text{-}24h}$, AUC from 0 to 24 h postdose; $AUC_{0\text{-}t}$, AUC from 0 to the time of last measurable concentration; $AUC_{0\text{-}inf}$, AUC from time 0 to extrapolated infinity; CI, confidence interval; C_{max}, maximum observed concentration; lova, lovastatin; LS, least squares; n, number of observations; NA, not available; PK, pharmacokinetics; Simva, simvastatin; T_{max}, time to maximum observed concentration.

14.2.5.2. Study NB4-01-06-019

Title

Assessment of Pharmacokinetic Drug-Drug Interaction between SD-5613, Simvastatin and Lovastatin after Oral Administration of Multiple Concomitant Doses in Healthy Volunteers

Objectives

To characterize the potential interaction of simvastatin and lovastatin when given concomitantly with maralixibat.

Study Design

This was a single center, open-label, randomized, multiple-dose, two-sequence, four-period, four-treatment, add-on design study in 24 healthy adult subjects. Subjects were screened for eligibility during a pretreatment period, which was up to 21 days prior to first study drug dose on Day 1. The treatment period extended for 28 days and began the evening prior to the first administration of study drug (Day -1). Subjects were randomized to two treatment sequences with four periods in each sequence and received all of the following four treatments during treatment periods I to IV. There was a 7-day washout period between treatment periods II and III.

- Treatment A: simvastatin 20 mg (alone)
- Treatment B: SD-5613 5 mg + simvastatin 20 mg
- Treatment C: lovastatin 20 mg (alone)
- Treatment D: SD-5613 5 mg + lovastatin 20 mg

Statins (simvastatin, 20 mg or lovastatin 20 mg) were dosed once daily at evening meal time, while maralixibat (5 mg once daily) was administered prior to breakfast on each of the dosing days.

Blood samples were collected prior to dosing on Days 3 and 4 in each treatment period, for measurement of statin (treatment periods I to IV). On Day 4 of treatment periods I and III (statin alone), and Day 5 of treatment periods II and IV (statin and maralixibat coadministration), blood samples were collected for the determination of statin and metabolite plasma concentrations prior to dosing of statin (predose; 0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after dosing. On Day 5 of treatment periods II and IV (statin and maralixibat coadministration), blood samples were collected for determination of maralixibat free-form plasma concentrations prior to dosing of maralixibat (predose; 0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after dosing.

PK Results

Maralixibat plasma concentrations were below the assay sensitivity limit at nearly all timepoints after administration of 5 mg maralixibat. Maralixibat did not have a clinically significant effect on the PK of simvastatin, after staggered administration (Table 118). A 17% average decrease in $AUC_{0\text{-}24h}$ and 10% average decrease in C_{max} were observed for the inactive parent compound (simvastatin; inactive lactone form). However, no effect was observed on the bioavailability ($AUC_{0\text{-}24h}$ and C_{max}) of its active metabolite, β-hydroxysimvastatin, although a delay was

observed in T_{max} (median 2 h). The means (90% CIs) of test to reference treatment ratios for simvastatin AUC_{0-24} and for C_{max} were 0.83 (0.75, 0.91) and 0.90 (0.77, 1.05), respectively (Table 118). The means (90% CIs) of test to reference treatment ratios for β-hydroxysimvastatin AUC_{0-24} and C_{max} were 0.96 (0.83, 1.11) and 0.95 (0.85, 1.07), respectively.

However, the dose of maralixibat (5 mg) used in this study is five-fold lower than the highest dose (400 mcg/kg/day equivalent to ~28 mg for a 70 kg adult). Although there are literature reports that support a role for OATP2B1 in the absorption of statins, the clinical implication of the change in simvastatin exposures at the proposed dosing regimen is unclear. As a result, we cannot rule out the need for titration of statin doses if coadministered with maralixibat in patients with ALGS.

Table 118. Lovastatin, β-Hydroxylovastatin, Simvastatin, and β-Hydroxysimvastatin PK Parameters in the Presence and Absence of Maralixibat

		LS Mean			
Analyte	Parameter	Test (n=24)	Reference (n=24)	Ratio of Test/Reference	90% CI
Simvastatin	AUC_{0-24h} (ng•h/mL)	18.55	22.49	0.83	0.75, 0.91
	C_{max} (ng/mL)	6.55	7.28	0.90	0.77, 1.05
β-hydroxysimvastatin	AUC_{0-24h} (ng•h/mL)	11.181	11.662	0.96	0.83, 1.11
	C_{max} (ng/mL)	1.08	1.14	0.95	0.85, 1.07
Lovastatin	AUC_{0-24h} (ng•h/mL)	21.10	21.69	0.97	0.88, 1.08
	C_{max} (ng/mL)	4.20	4.54	0.93	0.78, 1.10
β-hydroxylovastatin	AUC_{0-24h} (ng•h/mL)	25.85	23.40	1.11	0.97, 1.26
	C_{max} (ng/mL)	2.95	2.97	0.99	0.86, 1.15

Source: Clinical study report for NB4-01-06-019.
Test was the treatment of simvastatin or lovastatin in the presence of maralix bat; reference was treatment of simvastatin or lovastatin alone.
[1] n=19
[2] n=23
Abbreviations: AUC_{0-24h}, area under the curve from time 0 to 24 h; CI, confidence interval; C_{max}, maximum concentration; LS, least squares; n, number of observations; PK, pharmacokinetics

14.2.5.3. Study NB4-02-06-020

Title

Assessment of Pharmacokinetic Drug-Drug Interaction between Multiple Concomitant Doses of SD-5613 and Atorvastatin Administered Once Daily in the Morning Versus Once Daily in the Evening in Healthy Volunteers

Objective

The primary objective of this study was to characterize the potential for steady-state PK interaction between atorvastatin administered in the morning or evening, and concomitant multiple doses of maralixibat administered in the morning.

Study Design

This was a single center, open-label, randomized, multiple-dose, two-sequence, four-period, four-treatment, add-on design study in 24 healthy adult subjects. Subjects were screened for eligibility during a pretreatment period, which was up to 21 days prior to first study drug dose on Day 1. The treatment period extended for 28 days and began the evening prior to the first administration of study drug (Day -1). Subjects were randomized to two treatment sequences

with four periods in each sequence and received all of the following four treatments during treatment periods I to IV. Treatment A: atorvastatin 20 mg (alone) once daily in the morning for 5 days; treatment B: maralixibat 5 mg once daily in the morning + atorvastatin 20 mg once daily in the morning for 5 days; treatment C: atorvastatin 20 mg (alone) once daily in the evening for 5 days; and treatment D: maralixibat 5 mg once daily in the morning + atorvastatin 20 mg once daily in the evening for 5 days. There was a 7-day washout period between treatment periods II and III.

Blood samples were collected prior to dosing on Days 3 and 4 in each treatment period, for measurement of atorvastatin (treatment periods I to IV). On Day 4 of treatment C, blood samples were collected for the determination of statin and metabolite plasma concentrations prior to dosing of statin (predose; 0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after dosing. On Day 5 of treatment periods II and IV (statin and maralixibat coadministration), blood samples were collected for determination of maralixibat free-form plasma concentrations prior to dosing of maralixibat (predose; 0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after dosing.

PK Results

Coadministration of maralixibat with atorvastatin in the morning resulted in a modest decrease in steady-state atorvastatin and o-hydroxyatorvastatin exposure (17% mean decrease in atorvastatin $AUC_{0\text{-}24h}$ and 10% mean decrease in o-hydroxyatorvastatin $AUC_{0\text{-}24h}$, Table 119). Peak concentrations of atorvastatin did not appear to be affected by concomitant administration of maralixibat, but peak concentrations of o-hydroxyatorvastatin decreased modestly (12% mean decrease in o-hydroxyatorvastatin C_{max}) following morning administration. Atorvastatin and o-hydroxyatorvastatin median T_{max} values were comparable between morning treatments, with and without maralixibat. A definitive conclusion cannot be made with regard to p-hydroxyatorvastatin, since many plasma profiles were variable or below the LLOQ (<0.25 ng/mL).

For the evening treatments, coadministration of maralixibat with atorvastatin resulted in a modest decrease in atorvastatin exposure and a slight increase in o-hydroxyatorvastatin exposure (Table 120). Following concomitant administration, the atorvastatin $AUC_{0\text{-}24h}$ decreased by 14% on average, but o-hydroxyatorvastatin $AUC_{0\text{-}24h}$ increased by 8% on average, after evening dosing. Atorvastatin C_{max} showed an equally modest decrease (mean 14%) in the presence of maralixibat but no notable change was seen in the o-hydroxyatorvastatin C_{max}.

Table 119. Comparison of Atorvastatin, Ortho-Hydroxyatorvastatin, and Para-Hydroxyatorvastatin Pharmacokinetic Parameters Following Morning Administration of Atorvastatin Alone or in Combination With Maralixibat

Analyte	Least Squares Mean[a]				Comparison Test/Reference[b]		
	Test[c]		Reference[c]				
Parameter	N	Mean	N	Mean	Ratio	90% CI	P-value
Atorvastatin							
$AUC_{0\text{-}24h}$ (h•ng/mL)	19	42.36	23	51.27	0.83	0.77, 0.89	<0.001
C_{max} (ng/mL)	19	8.44	23	8.88	0.95	0.81, 1.12	0.600
o-Hydroxyatorvastatin							
$AUC_{0\text{-}24h}$ (h•ng/mL)	19	43.17	23	48.25	0.90	0.81, 0.99	0.081
C_{max} (ng/mL)	19	4.03	23	4.56	0.88	0.73, 1.07	0.271
p-Pydroxyatorvastatin							
$AUC_{0\text{-}24h}$ (h•ng/mL)	13	5.89	17	6.60	0.89	0.69, 1.16	0.449
C_{max} (ng/mL)	13	0.45	17	0.51	0.88	0.76, 1.02	0.159

Source: Table 6 from the clinical study report for NB-02-06-020.
Results of ANOVA of natural log-transformed pharmacokinetic parameters, using a model accounting for subject and treatment effects.
[a] Least-squares means are back-transformed to the original scale.
[b] Ratio of least-squares means and 90% confidence intervals for comparison of test to reference treatment, and the p-value for treatment effect from the ANOVA model.
[c] Test: SD-5613 5 mg once daily (AM) + atorvastatin 20 mg once daily (AM); Reference: atorvastatin 20 mg once daily (AM).
Abbreviations: ANOVA, analysis of variance; $AUC_{0\text{-}24h}$, area under the curve from time 0 to 24 h; CI, confidence interval; C_{max}, maximum concentration

Table 120. Atorvastatin, Ortho-Hydroxyatorvastatin and Para-Hydroxyatorvastatin Pharmacokinetic Parameters Following Evening Administration of Atorvastatin Alone or in Combination With Maralixibat

Analyte	Least-Squares Means[a]				Comparison Test/Reference[b]		
	Test[c]		Reference[c]				
Parameter	N	Mean	N	Mean	Ratio	90% CI	P-value
Atorvastatin							
$AUC_{0\text{-}24h}$ (h•ng/mL)	20	39.20	21	45.42	0.86	0.66, 1.13	0.359
C_{max} (ng/mL)	20	6.66	21	7.70	0.86	0.61, 1.23	0.478
o-Hydroxyatorvastatin							
$AUC_{0\text{-}24h}$ (h•ng/mL)	20	38.84	21	36.14	1.08	0.98, 1.19	0.218
C_{max} (ng/mL)	20	3.03	21	2.98	1.02	0.82, 1.27	0.893
p-Hydroxyatorvastatin							
$AUC_{0\text{-}24h}$ (h•ng/mL)	16	5.54	14	3.13	1.77	1.22, 2.57	0.018
C_{max} (ng/mL)	16	0.52	14	0.46	1.13	0.90, 1.42	0.350

Source: Table 7 from the clinical study report for NB-02-06-020.
Results of ANOVA of natural log transformed pharmacokinetic parameters, using a model accounting for subject and treatment effects.
[a] Least-squares means are back-transformed to the original scale.
[b] Ratio of least-squares means and 90% confidence intervals for comparison of test to reference treatment, and the p-value for treatment effect from the ANOVA model.
[c] Test: SD-5613 5 mg once daily (AM) + atorvastatin 20 mg once daily (AM); reference: atorvastatin 20 mg once daily (AM).
Abbreviations: ANOVA, analysis of variance; $AUC_{0\text{-}24h}$, area under the curve from time 0 to 24 h; CI, confidence interval; C_{max}, maximum concentration

14.2.6. Studies in Pediatric Patients (LUM001-301, LUM001-302, LUM001-303, LUM001-304, LUM001-305, LUM001-302, MRX-EAP)

The Applicant has conducted three multicenter, randomized, placebo-controlled studies (Studies LUM001-301, LUM001-302, LUM-304) and single-arm, long-term extension studies (Studies LUM001-305 and LUM001-303). Doses lower than the proposed dose of 400 mcg/kg/day (i.e., 380 mcg/kg/day, free-base equivalent) were studied in two placebo-controlled dose-ranging

studies (LUM001-301 and LUM001-302). In addition, the Applicant has provided results from their expanded access program (MRX-EAP). Table 121 lists the design elements for all clinical pharmacology-related studies in pediatric participants with ALGS.

Table 121. Completed Clinical Pharmacology-Related Studies in Pediatric Subjects With ALGS

Study	Study Objective	Study Design	Subject Entered/ Completed; Sex; Age	Treatment	Dosing* (µg/kg/day)	Subject of Active Dosing Group
LUM001-301	Safety and efficacy	Double-blind, randomized, placebo controlled, parallel group in pediatric patients 1-18 yrs of age with ALGS	37/35; 21M/16F; 1-17 yrs	Maralixibat 70, 140, and 280 µg/kg/day for 13 weeks (n=6-11 each dose, 25 total); placebo (n=12)	70 QD	8
					140 QD	11
					280 QD	6
LUM001-302	Safety and efficacy	Double-blind, placebo-controlled, in pediatric patients 1-18 yrs of age with ALGS	20/19; 10M/10F; 1-16 yrs	Maralixibat 140 and 280 µg/kg/day for 13 weeks (n=6-8 each dose, 14 total); placebo (n=6)	140 QD	6
					280 QD	8
LUM001-303	Long-term safety and efficacy	OL in ALGS	19/6, 10M/9F; 1-16 yrs	Maralixibat 280 µg/kg/day QD, increased to BID	280 QD	19
					280 BID	5
LUM001-304	Safety and efficacy	Randomized, placebo-controlled, drug-withdrawal study with an OL extension in children with ALGS	31(entered)/ 29 (randomized); 19M/12F; 1-15 yrs	Maralixibat 400 µg/kg/day QD (Week 0-18, n=31; Week 19-22, n=13; Week 48 onward [could increase to 400 µg/kg BID]: n=29)	400 QD, or 400 BID after Week 48	31
LUM001-305	Safety and efficacy	OL in ALGS	34/21, 20M/14F; 1-17 yrs	Maralixibat 280 µg/kg/day QD	280 QD	34

Source: Adapted from Table 16 Section 2.7.2. of the Summary of Clinical Pharmacology Studies.
* Dosing is based on the chloride salt form of maralixibat. The equivalent free-base doses are 66.5, 133, 266, and 380 µg/kg/day.
Abbreviations: ALGS, Alagille syndrome; BID, twice daily; F, female; M, male; OL, open-label; QD, once daily

14.2.6.1. Study LUM001-301

Title

ITCH Study: Evaluation of the Intestinal Bile Acid Transport (IBAT) Inhibitor LUM001 in the Reduction of Pruritus in Alagille Syndrome, a Cholestatic Liver Disease

Objectives

The objectives of this study were:

- To evaluate the effect of maralixibat versus placebo on pruritus as measured by the Itch Reported Outcome (ItchRO) instrument.
- To evaluate the safety and tolerability of maralixibat.
- To evaluate the effect of maralixibat versus placebo on sBA.
- To explore the effect of maralixibat versus placebo on other biochemical markers of cholestasis and liver disease.

Study Design

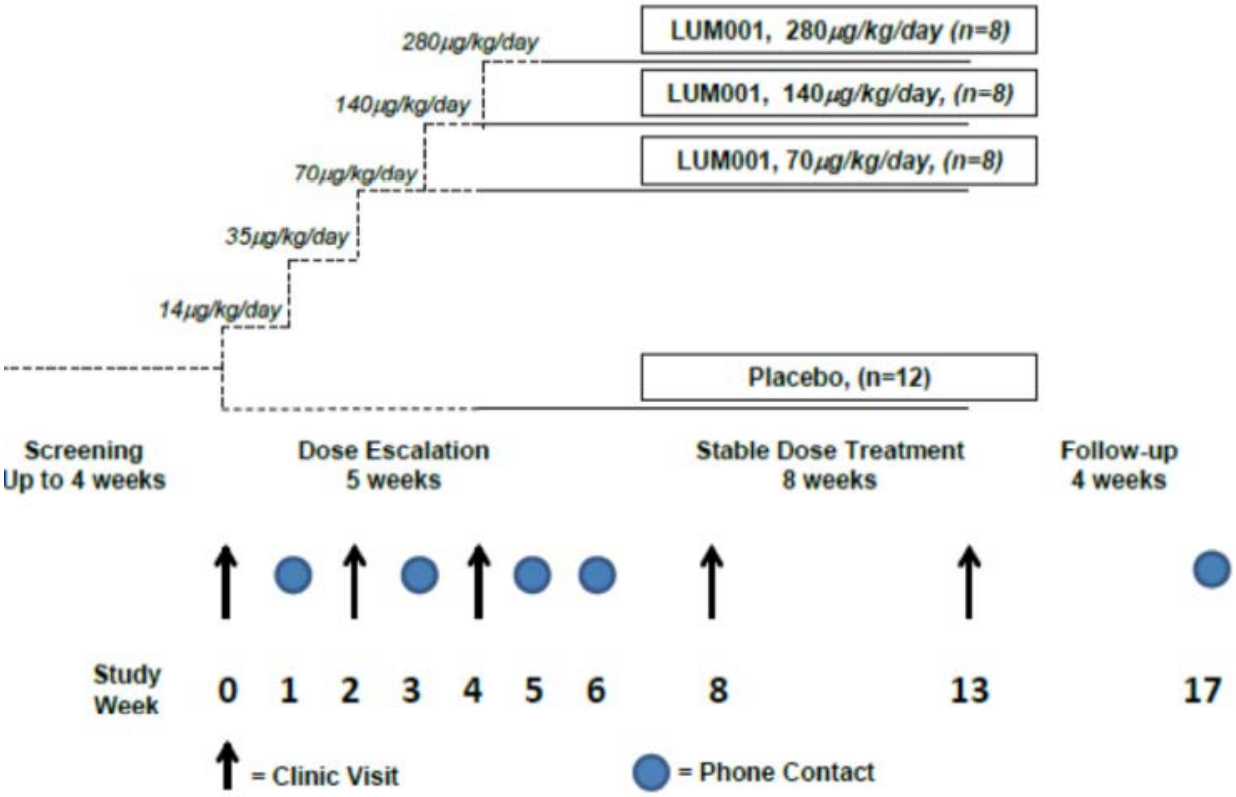
This was a randomized, double-blind, placebo-controlled, parallel group, multicenter study in children with ALGS (age 12 months to 18 years) (Figure 21). The study was designed to investigate the effects of maralixibat, compared to placebo, following daily dosing over a 13-week period.

Eligible subjects with ALGS were randomly assigned to one of the four treatment groups:

- Maralixibat low dose: 70 µg/kg/day maralixibat chloride (maximum daily dose of 5 mg/day) (n=8)
- Maralixibat mid dose: 140 µg/kg/day maralixibat chloride (maximum daily dose of 10 mg/day) (n=8)
- Maralixibat high dose: 280 µg/kg/day maralixibat chloride (maximum daily dose of 20 mg/day) (n=8)
- Placebo (n=12)

There was a 2:1 randomization ratio between maralixibat and placebo. Approximately 36 evaluable subjects were planned for enrollment into this study; 37 subjects were enrolled and analyzed. Plasma PK samples were collected at 2 h postdose at Week 4 and at 4 h postdose at Weeks 2, 8, and 13.

Figure 21. Study Design Schematic



Source: Clinical study report for LUM001-301
Abbreviations: LUM001, maralixibat

PK Results

Plasma maralixibat concentrations at 2 or 4 h postdose were mostly below the LLOQ (0.25 ng/mL).

PD Results

Serum Bile Acid Levels

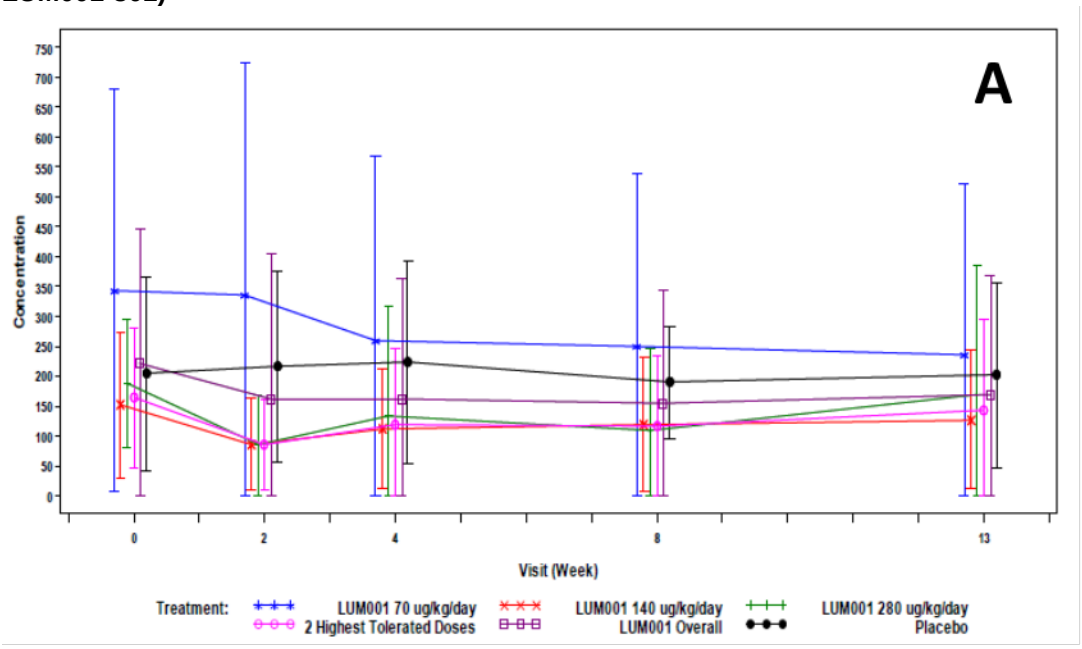
The change from baseline in sBA levels was highly variable. The sBA levels at baseline were highly variable within and across treatment groups. For example, in 280 mcg/kg cohort, two patients had sBAs <100 mcmol/L at baseline and their sBAs did not change by maralixibat. The treatment effect in the 70 µg/kg/day group was driven primarily by one outlier with a very high baseline sBA level and a large change over the course of the study, which could be due to natural variability rather than a true treatment effect. Further, there were no concomitant changes in serum C4 levels (a marker of intrahepatic bile acid synthesis, upregulated by ASBT inhibition), suggesting a natural variability of sBA rather than a true treatment effect. See Table 122 and Figure 22.

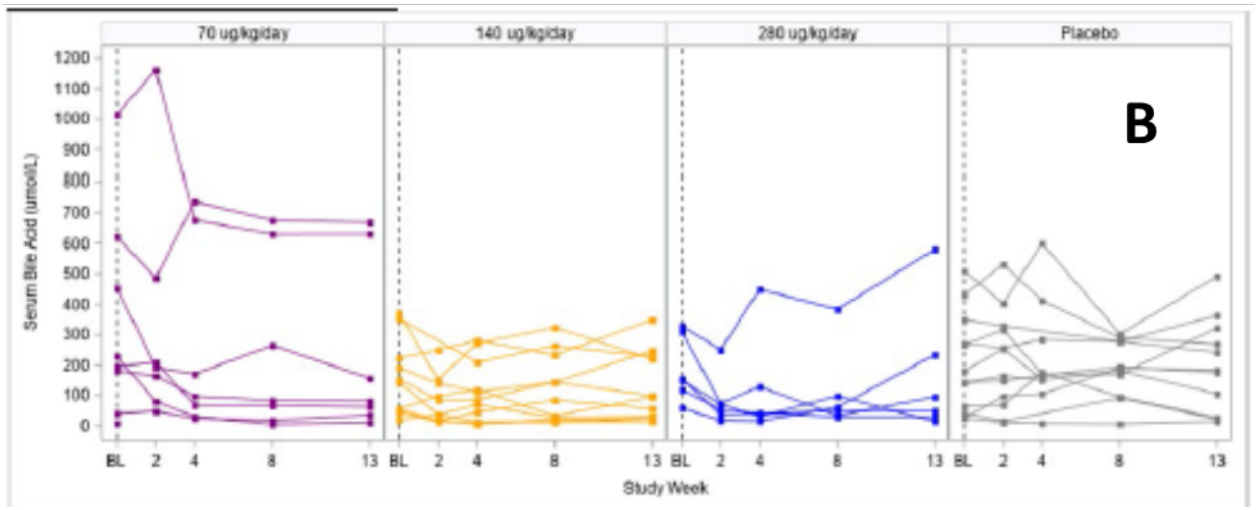
Table 122. Primary Analysis of Secondary Endpoints: Total Serum Bile Acid Level Change From Baseline to Endpoint (Week 13/ET)—Tabulation of Fitted Summary Statistics From ANCOVA (Modified Intent-to-Treat Population)

Variable	Maralixibat (µg/kg/day)					
Statistic[a]	70 (N=8)	140 (N=11)	280 (N=6)	2 Highest Tolerated[b] (N=17)	Overall (N=25)	Placebo (N=12)
Serum Bile Acid (µmol/L)						
LS Mean (SE)	-117.401 (46.2353)	-40.358 (34.8650)	-27.437 (46.2823)	-33.898 (29.1984)	-61.732 (23.9476)	-10.442 (32.6776)
(95% CI for LS Mean)	(-211.699, -23.103)	(-111.466, 30.749)	(-121.831, 66.956)	(-93.448, 25.653)	(-110.574, -12.891)	(-77.089, 56.204)
p-value (LS Mean=0)	0.0163	0.2559	0.5576	0.2545	0.0149	0.7514
LS Mean Difference from Placebo (SE)	-106.959 (57.1714)	-29.916 (47.5096)	-16.995 (56.5432)	-23.455 (43.5991)	-51.290 (40.6120)	
(95% CI for Mean Difference from Placebo)	(-223.560, 9.643)	(-126.812, 66.980)	(-132.316, 98.326)	(-112.376, 65.466)	(-134.119, 31.539)	
p-value (Maralixibat LS Mean=Placebo LS Mean)	0.0708	0.5335	0.7658	0.5944	0.2160	

Source: Table 19 in the clinical study report for LUM001-301.
[a] Estimates are from a mixed model with treatment group as a fixed effect and baseline value as a covariate. Maralixibat treatment groups (combined and individual) were tested against the placebo group.
[b] The "2 Highest Tolerated Doses" active dose group (maralixibat-2HDC) combines the two highest active dose groups that meet the tolerability criteria (i.e., ≥50% of the subjects in the dose group tolerate the treatment). These two treatments are: maralix bat 140 and 280 µg/kg/day. The first test performed for each outcome measure is a comparison of the maralixibat-2HDC dose group and placebo. Assigned/planned treatment groups are presented. Endpoint (Week 13/ET) represents the last postbaseline value obtained within 7 days of the date of last dose.
Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval, ET, end of treatment; LS, least-squares; N, number in population; SE, standard error

Figure 22. Serum BA Concentrations From Baseline to Week 13/ET by Treatment Group: Panel A, Average Values of the Treatment Groups. Panel B, Individual Values Across Treatment Groups (Study LUM001-301)



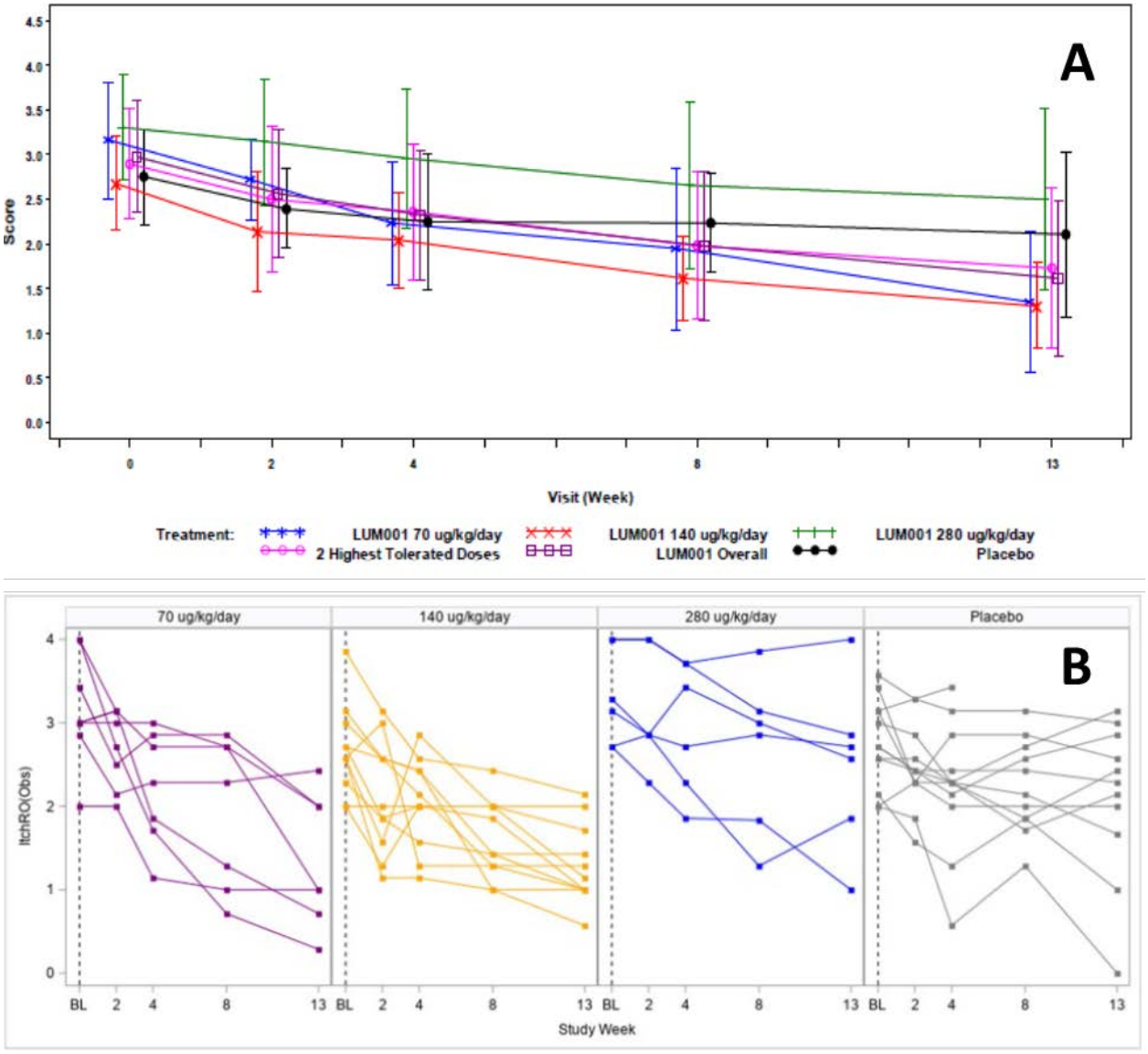


Source: Clinical study report for LUM301 and Appendix 2 from the Summary of Clinical Efficacy.
Abbreviations: BA, bile acids; ET, end of treatment; LUM001, maralix bat

Pruritus Assessment

The primary efficacy endpoint comparing the ItchRO score in the two higher-dose maralixibat groups combined (140 and 280 µg/kg/day) with the placebo group at Week 13/ET was not met. The placebo-adjusted Itch-Reported Outcome (Observer) (ItchRO(Obs)) weekly average (based on daily maximum) severity score least squares (LS) mean (standard error) reduction for participants receiving maralixibat was -0.473 (0.33) and did not reach statistical significance (p=0.1594). There was a trend toward pruritus reduction was observed in the maralixibat treated groups (Figure 23) although a dose-relationship was not observed.

Figure 23. ItchRO(Obs) Weekly Average Severity Score Over Time: Panel A, Average Values of the Treatment Groups. Panel B, Individual Values Across the Treatment Groups (Study LUM001-301)



Source: Clinical study report for LUM301 and Appendix 2 of the Summary of Clinical Efficacy.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); LUM001, maralixibat

Changes in Other Exploratory Biochemical Markers (C4, Fibroblast Growth Factor-19, and Autotaxin)

There appeared to be a numerical increase from baseline in C4 levels in all maralixibat treatment groups, although none was statistically significant. Similarly, there were no statistically significant changes in fibroblast growth factor-19 (FGF-19) in the active treatment groups over the course of the study. There were numerical decreases in the 70 and 280 µg/kg/day groups and a numerical increase in the 140 µg/kg/day group. No change from baseline or difference from placebo reached statistical significance and a dose-response relationship was not evident. In the case of autotaxin, there was no consistent change from baseline in the active treatment groups with decreases observed in the 70 and 140 µg/kg/day dose groups and an increase in the 280 µg/kg/day dose group (Table 123).

Table 123. Changes in C4, FGF-19, and Autotaxin Levels (Difference From Placebo) at Week 13 (Study LUM001-301)

Factor	70 µg/kg N=8	140 µg/kg N=11	280µg/kg N=6
C4 (ng/mL)	6.71 (7.210)	10.10 (6.302)	11.06 (7.359)
FGF-19 (pg/mL)	96.13 (183.667)	291.99 (158.369)	-41.35 (187.089)
Autotaxin (ng/mL)	-1156.24 (672.480)	-804.99 (602.095)	389.55 (744.336)

Source: Reviewer, based on data from Tables 40, 41, and 42 in the clinical study report for LUM001-301.
Values are LS mean differences from placebo (SE).
Abbreviations: C, complement; FGF, f broblast growth factor; LS, least squares; SE, standard error;

14.2.6.2. Study LUM001-302

Title

IMAGO Study: Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of LUM001, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi), in the Treatment of Cholestatic Liver Disease in Pediatric Patients with Alagille Syndrome.

Objectives

To evaluate (a) the safety and tolerability of maralixibat in pediatric subjects with ALGS, (b) the effect of maralixibat versus placebo on sBA associated with ALGS, (c) the effect of maralixibat versus placebo on liver enzymes associated with ALGS, (d) the effect of maralixibat versus placebo on pruritus associated with ALGS, and (e) the effect of maralixibat versus placebo on other biochemical markers associated with ALGS.

Study Design

This was a randomized, double-blind, placebo-controlled, Phase 2 proof-of-concept and safety study in children with ALGS. The study was designed to investigate the effects of maralixibat, compared with placebo, on sBA, liver enzymes, pruritus, and other biochemical markers associated with cholestatic liver disease, following daily dosing over a 13-week period.

Two dose cohorts were planned. In each cohort, eligible subjects were randomized in a ratio of 2:1 to receive active drug or a placebo. Enrollment into Cohort A was completed prior to enrollment of subjects into Cohort B.

- Cohort A: 140 µg/kg/day maralixibat (n=6), or placebo (n=3)
- Cohort B: 70 or 280 µg/kg/day maralixibat (n=6), or placebo (n=3)

Plasma PK samples were collected at baseline and at approximately 4 h postdose at Week 5 and Week 13/end of treatment (ET).

PK Results

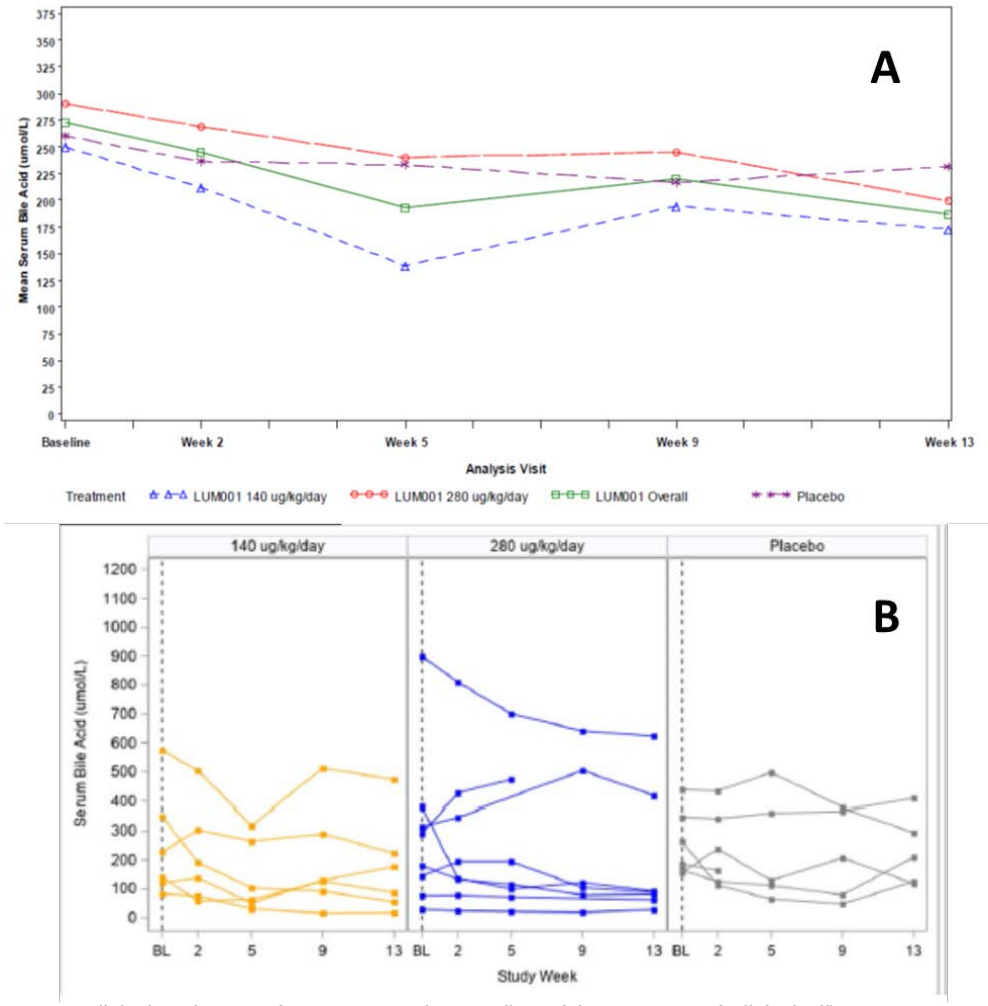
Only one sample, obtained from a patient treated with 280 µg/kg/day maralixibat, had a maralixibat concentration above the assay limit of detection (0.25 ng/mL); this sample was collected at Week 13/ET at 6.75 h postdose and had a concentration of 0.304 ng/mL. All other samples were below the LLOQ.

PD Results

Serum Bile Acid Levels

The primary endpoint was the change from baseline in sBA at Week 13/ET. The sBA levels were highly variable among patients. There was no major change in mean sBA levels relative to baseline observed in the treatment groups at Week 13/ET (Figure 24). A dose-response relationship was not apparent. The LS mean (standard error) differences from baseline values were -49.4 (43.5) µmol/L, -82.9 (50.2) µmol/L, and -42.2 (50.1) µmol/L for the 280 µg/kg/day, 140 µg/kg/day , and placebo group, respectively.

Figure 24. Serum BA Concentrations from Baseline to Week 13/ET by Treatment Group (Panel A) and by Individual (Panel B) (Study LUM001-302)



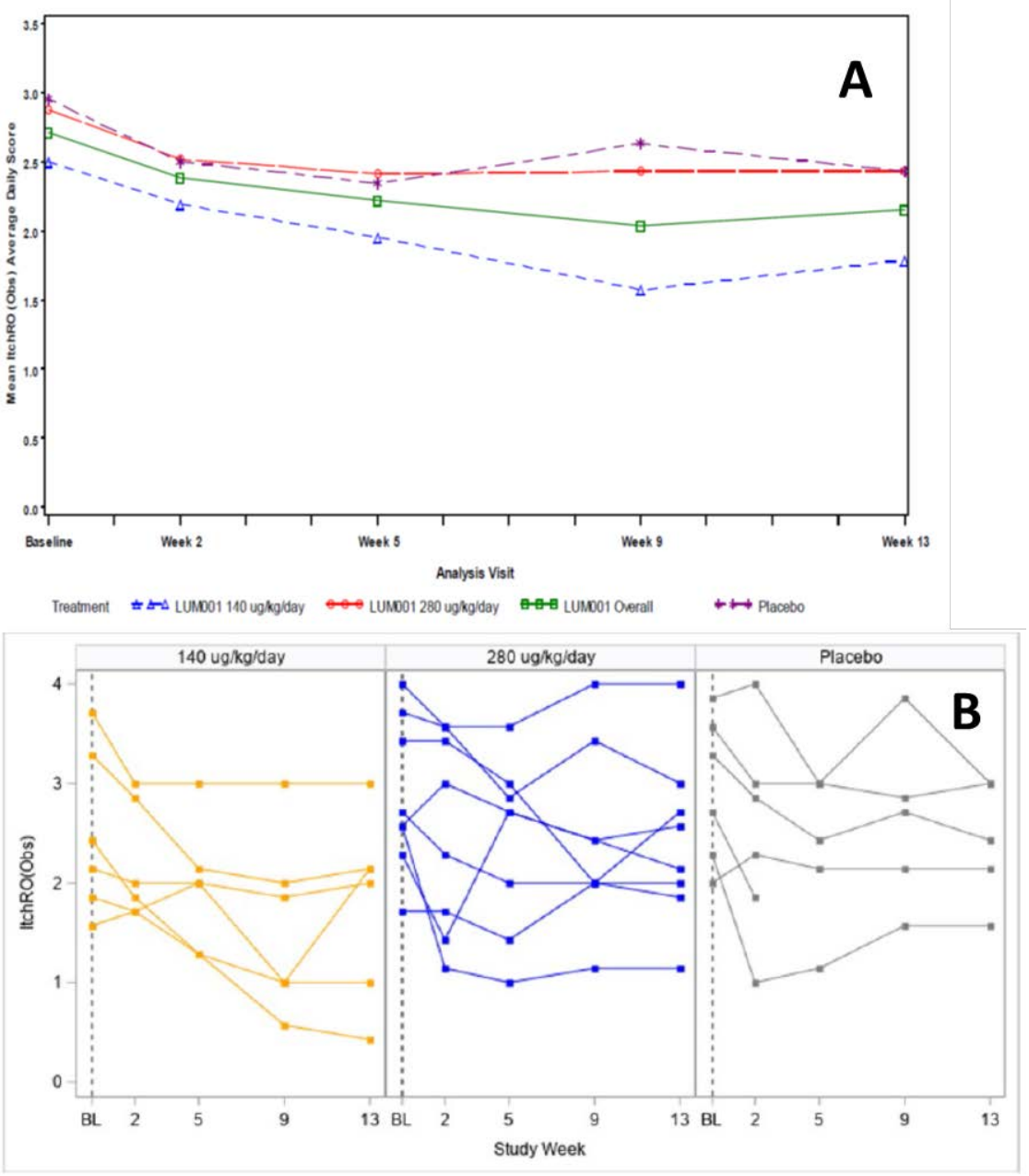
Source: Clinical study report for LUM302 and Appendix 3 of the Summary of Clinical Efficacy.
Abbreviations: BA, bile acids; ET, end of treatment; LUM001, maralix bat

Pruritus Assessment

No significant differences in the ItchRO(Obs) weekly average severity scores (based on daily maximum) were seen for the maralixibat 140 µg/kg/day or 280 µg/kg/day compared with the placebo group (Figure 25). Decreases in ItchRO(Obs) weekly average severity scores (LS mean

change [SE]) from baseline values to the Week 13/ET visit were noted in the maralixibat 140 µg/kg/day treatment group (-0.802 [0.2732]) as well as in the placebo group of -0.592 (0.2690).

Figure 25. ItchRO (Obs) Weekly Average Severity Score Over Time: Panel A, Average Values of the Treatment Groups. Panel B, Individual Values Across Treatment Groups (Study LUM001-302)



Source: Clinical study report for LUM302 and Appendix 3 of the Summary of Clinical Efficacy.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); LUM001, maralixibat

Changes in Other Exploratory Biochemical Markers (Serum C4 Level)

At Week 13, serum 7αC4 levels were not significantly different compared with placebo in both treatment groups.

14.2.6.3. Study LUM001-303

Title

IMAGINE Study: Multicenter Extension Study to Evaluate the Long-Term Safety and Durability of the Therapeutic Effect of LUM001, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi), in the Treatment of Cholestatic Liver Disease in Pediatric Subjects with Alagille Syndrome

Objectives

The primary objective of the study (up to and including Week 72) was to evaluate the long-term safety and tolerability of maralixibat in pediatric patients with ALGS.

The secondary objectives of the study (up to and including Week 72) were as follows:

- To evaluate the long-term effect of maralixibat on sBA.
- To evaluate the long-term effect of maralixibat on pruritus.
- To explore the long-term effect of maralixibat on other biochemical markers of cholestasis and liver disease.
- To evaluate the long-term effect of maralixibat on xanthomas.
- To explore an expanded dosing range to identify the doses necessary to achieve the optimal benefit-to-risk ratio for this patient population.

The objectives of the long-term follow-up treatment period for participants who were eligible for Protocol Amendment 5 were as follows:

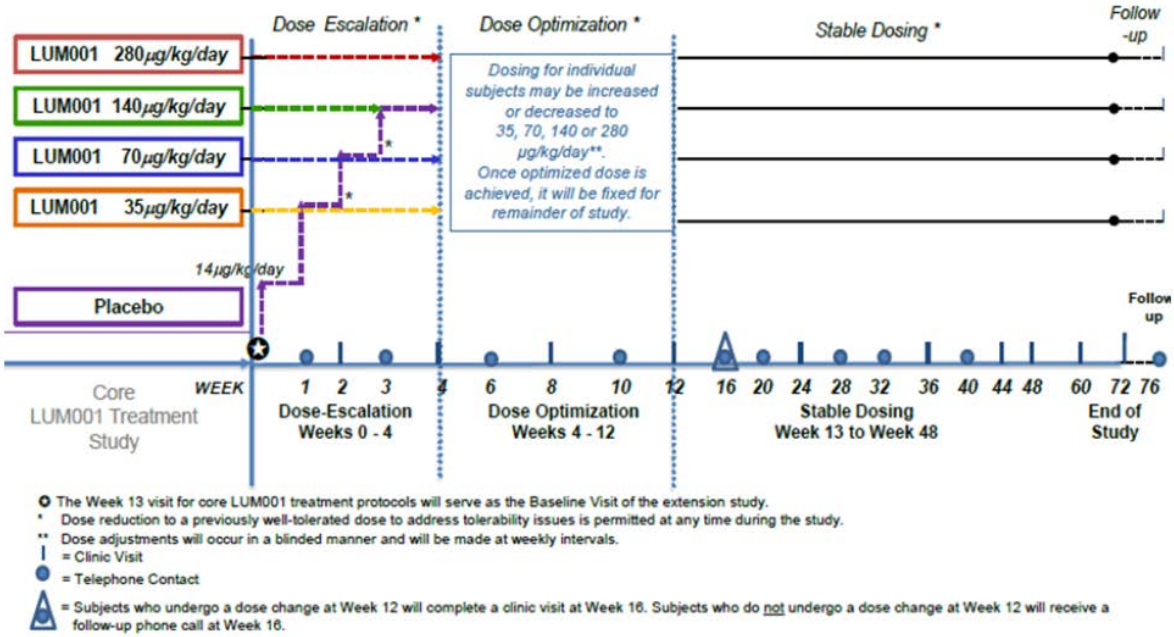
- To explore a twice daily (BID) dosing regimen and higher daily dosing of maralixibat.
- To obtain safety and efficacy data in participants treated long-term on maralixibat.
- To assess the level of AFP, a marker of hepatocellular carcinoma.

Study Design

This was a multicenter, double-blind study of maralixibat in children ≥12 months of age diagnosed with ALGS who completed participation in Study LUM001-302. All participants received maralixibat in Study LUM001-303. See Figure 26.

The study was divided into five parts: 1) a dose-escalation period, 2) a dose-optimization period, 3) a stable dosing period, 4) a 52-week follow-up period, and 5) a long-term follow-up treatment period for eligible participants whose caregivers chose for them to stay on treatment with maralixibat. During this long-term follow-up treatment period, participants may have had their dose of maralixibat increased to a maximum of 560 µg/kg/day (280 µg/kg twice daily [BID]), based on efficacy (sBA level and ItchRO[Obs] score) and safety assessments.

Figure 26. Design of Study LUM001-303



Source: Clinical study report for LUM001-303
Abbreviations: DE, dose escalation; LUM001, maralixibat

Dose Optimization and Escalation Scheme

All participants entering the extension study participated in a 4-week double-blind dose-escalation period, during which participants who were randomized to receive placebo during Study LUM001-302 received weekly dose increases of maralixibat up to a target dose of 140 µg/kg/day or to a maximum tolerated dose below 140 µg/kg/day (10 mg maximum total dose). Participants who were randomized to receive active drug during Study LUM001-302 continued to receive the dose of maralixibat that they were taking at Week 13 of Study LUM001-302. A minimum of 7 days was required to elapse between dose increases.

After completion of the 4-week dose-escalation period, participants entered an 8-week dose optimization period. During this period, maralixibat dosing was adjusted with the objective of achieving optimal control of pruritus at a dose level that was tolerated by the participant and up to a maximum daily dose of 280 µg/kg maralixibat or 20 mg total dose.

Each participant received one of the following dose levels:

- Maralixibat 35 µg/kg/day.
- Maralixibat 70 µg/kg/day.
- Maralixibat 140 µg/kg/day.
- Maralixibat 280 µg/kg/day.

During the long-term follow-up treatment period, participants could have their dose of maralixibat increased to a maximum of 560 µg/kg/day (280 µg/kg BID), based on efficacy (sBA and ItchRO score) and safety assessment results.

PK Results

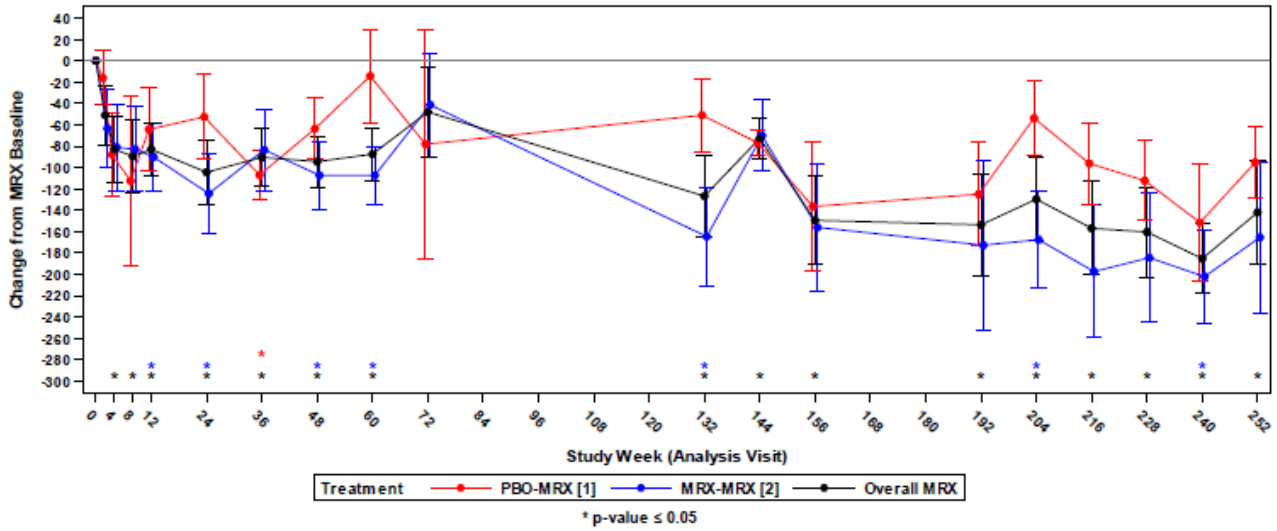
The vast majority of maralixibat drug levels were below the LLOQ (0.25 ng/mL). The highest drug level measured was 2.58 ng/mL (Subject (b) (6)).

PD Results

Serum Bile Acid Level

The primary efficacy endpoint in Study LUM001-303 was the change from baseline to Week 48 in fasting sBA level. Over the study duration, the mean improvement from maralixibat baseline in sBA in the overall population ranged from -47.89 µmol/L (Week 72) to -185.23 µmol/L (Week 240). (Figure 27).

Figure 27. Change in Serum BA Concentration from Baseline by Treatment Group Over Time (Study LUM001-303)

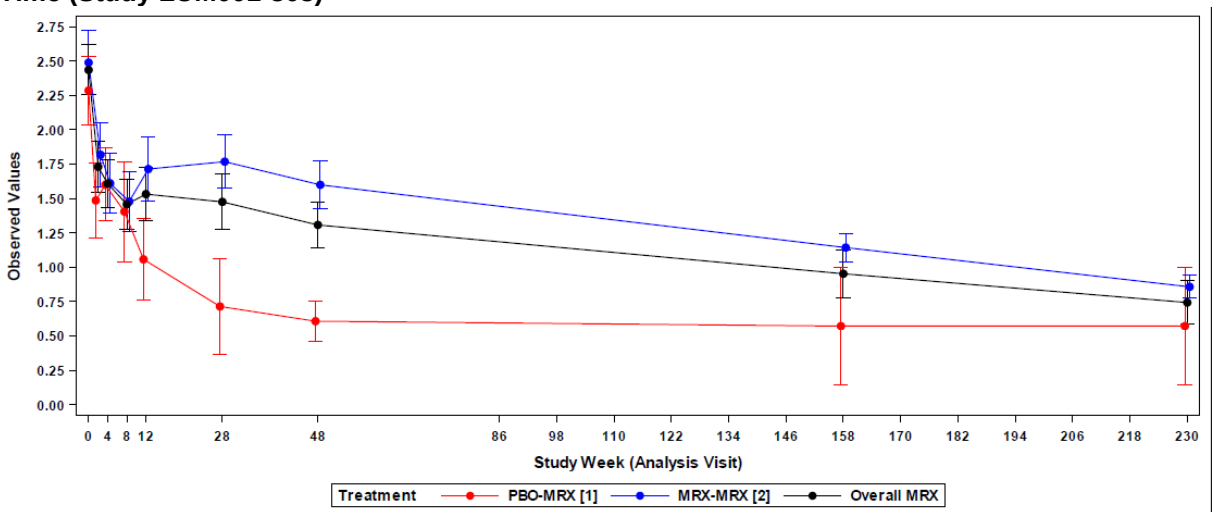


Source: Clinical study report for LUM303.
Abbreviations: BA, bile acids; MRX, maralixibat; PBO, placebo; SE, standard error

Pruritus Assessment

An improvement in pruritus was noted at all time points in the study from maralixibat baseline to Week 216, as measured by ItchRO(Obs) weekly average morning severity scores (Figure 28). At Week 48, a mean (SE) decrease (improvement) from maralixibat baseline in ItchRO(Obs) weekly average morning severity score (-1.095 [0.1740])) was observed. Over the duration of the study, the mean observed improvement from baseline in ItchRO(Obs) weekly average morning severity score in the overall population of open-label treatment continued to Week 230.

Figure 28. Mean (±SE) ItchRO(Obs) Weekly Average Morning Severity Score by Treatment Group Over Time (Study LUM001-303)

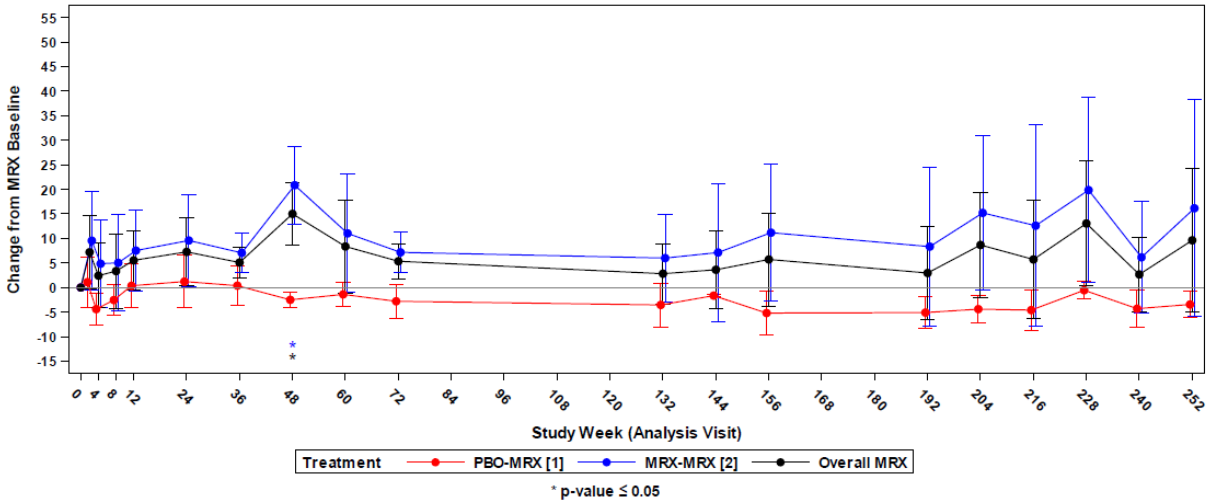


Source: Clinical study report for LUM001-303.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PBO, placebo; SE, standard error

Changes in Other Exploratory Biochemical Markers (Serum C4 Level)

In the overall population, the only mean (SD) change from maralixibat baseline in C4 was observed at Week 48 (15.01 [25.731] ng/mL). Over the duration of the study, mean increases from maralixibat baseline in C4 were observed from Week 2 to Week 252 (range 2.42 ng/mL [Week 4] to 15.01 ng/mL (Figure 29).

Figure 29. Mean (±SE) Change From Baseline in C4 (ng/mL) by Treatment Group Over Time (Study LUM001-303)



Source: Clinical study report for LUM001-303.
Abbreviations: C, complement; MRX, maralixibat; PBO, placebo; SE, standard error

14.2.6.4. Pivotal Study LUM001-304

Title

ICONIC Study: Long-Term, Open-Label Study with a Double-Blind, Placebo-Controlled, Randomized Drug Withdrawal Period of LUM001, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi), in Patients with Alagille Syndrome

Study Design

This was a randomized, placebo-controlled, drug-withdrawal study with a long-term open-label (OL) extension in children with ALGS designed to evaluate the safety and efficacy of maralixibat. The study comprised an 18-week OL run-in period (OL phase), a 4-week randomized, double-blind, placebo-controlled drug-withdrawal period (randomized withdrawal phase; RWD), a 26-week stable-dosing period at doses up to 400 µg/kg/day (after randomized withdrawal phase; ARW), and an optional long-term treatment period (long-term extension phase; LTE). During the long-term treatment period, participants may have had their dose of maralixibat increased to a maximum of 800 µg/kg/day (400 µg/kg twice daily), based on efficacy of sBA levels and ItchRO Observer [ItchRO(Obs)] score and safety assessments.

Participants continued in the optional long-term follow-up treatment period until the first of the following occurred: 1) the participants were eligible to enter another maralixibat study, 2) maralixibat was available commercially, or 3) the sponsor stopped the program or development in this indication. Refer to Section 15 for more details.

Table 124 summarizes drug exposure by study phase and by overall study population. In the overall study population, the treatment duration (SD) was 944.3 (587.07) days, with a mean average daily dose (SD) of 439.8 (133.47) µg/kg/day.

Table 124. Study Drug Exposure by Study Phase (Safety Population) (Study LUM001-304)

	Open-label Phase (Day 1 to Week 18)	Randomized Withdrawal Phase (Weeks >18 to 22)		ARW Phase (Weeks >22 to 48)	LTE Phase (Weeks >48)		Overall
	MRX QD	MRX QD	Placebo	MRX QD	MRX QD	MRX BID	MRX
Variable Statistic	N=31	N=13	N=16	N=29	N=23	N=15	N=31
Average Daily Dose (µg/kg/day)							
n	31	13	0	29	23	15	31
Mean	302.9	398.9	--	366.4	385.0	763.2	439.8
SD	48.38	3.96	--	29.20	15.21	64.29	133.47
Median	318.8	400.0	--	347.8	383.6	786.8	383.2

Source: Clinical study report for LUM001-304.
Abbreviations: ARW, after randomized withdrawal; BID, twice daily; LTE, long-term extension; MRX, maralixibat; QD, once daily; SD, standard deviation

Plasma samples for PK analysis were collected at baseline, and approximately 4 h postdosing at one additional time point (Week 12, 18, 38, or 48). In the follow-up treatment period afternoon dose escalation (ADE), PK samples were collected following completion of the ADE period.

PK Results

Due to the low systemic absorption of maralixibat, plasma maralixibat concentrations in most of the samples were below the LLOQ (0.250 ng/mL). The maximum measured value of 5.93 ng/mL was found at Week 12.

PD Results

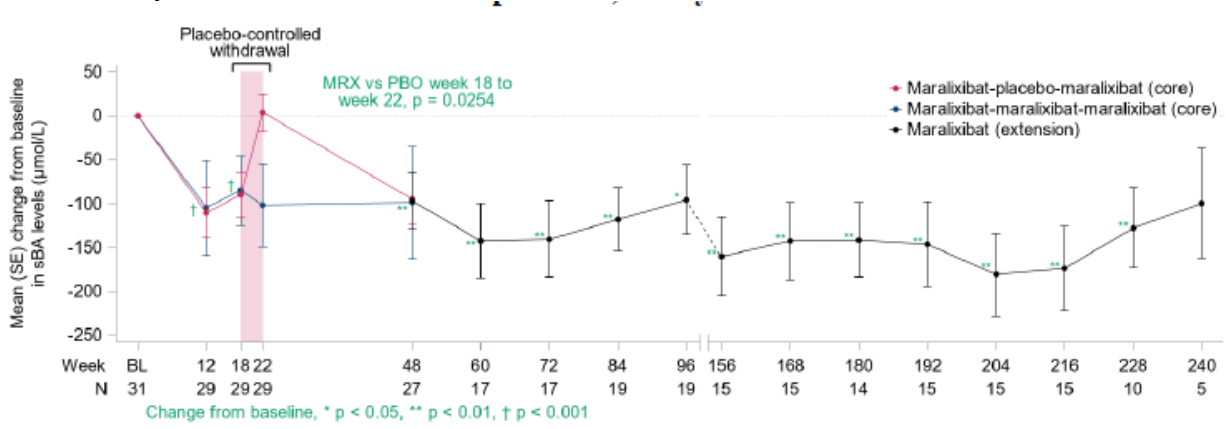
Serum Bile Acid Level

Total sBA concentrations were determined using a bioanalytical method validated in accordance with College of American Pathologists/Clinical Laboratory Improvement Amendments requirements. However, the bioanalytical method for sBA levels was not adequately validated according to the FDA Guidance for Industry Bioanalytical Method Validation . As a result, the reliability of the reported sBA values cannot be assured; therefore, the sBA results below should be considered exploratory and allow for assessment of overall trends in a general qualitative manner.

The Applicant assessed the change from Week 18 to Week 22 in fasting sBA levels in participants who had a reduction in sBA ≥50% from baseline to Week 12 or Week 18 (mITT population). Fifteen participants (5 assigned to the maralixibat group; 10 assigned to the placebo group during the RWD phase) met this prespecified sBA reduction criterion prior to the RWD phase. Participants administered placebo (n=10) during the RWD phase had a significant LS mean (SE) increase in sBA from Week 18 to Week 22 of 95.6 (30.5 µmol/L, whereas those who continued to receive maralixibat (n=5) had no notable increase (-21.7 [43.1] µmol/L).

The overall intent-to-treat population (n=31 participants), with all participants randomized to either placebo or maralixibat during the RWD phase, was used to analyze the effects of maralixibat on sBA over time. There was a significant mean (SE) decrease from baseline in sBA during the OL phases up to Week 18 (-87.7 [22.33] µmol/L, N=29), and Week 48 (-96.4 [32.1] µmol/L, N=27). Figure 30 shows sBA changes throughout the treatment period in the overall study population (ITT). Refer to Section 16 for comments on Pruritus Assessment.

Figure 30. Change in Serum BA Concentration From Baseline Over Time by Treatment Group (Study LUM001-304)



Source: Summary of clinical efficacy.
Abbreviations: BA, bile acids; BL, baseline; MRX, maralixibat; PBO, placebo; sBA, serum bile acids; SE, standard error

14.3. Population Pharmacokinetics

A population PK analysis was not provided by the Applicant. The Applicant indicated that since clinical studies in patients have only assessed plasma drug concentrations using sparse samples collected around the time of anticipated C_{max}, and the minimal absorption of maralixibat leads to plasma drug levels that are often below the LLOQ at therapeutic doses, PK parameters were not

calculable in patients and no population PK analysis was feasible. The Applicant's justification for the lack of a population PK analysis is acceptable.

14.4. Physiologically-based Pharmacokinetic Modeling (PBPK)

Background

Maralixibat is a (b) (4)

The proposed maralixibat starting dose is 200 μg/kg/day (190 μg/kg/day free base equivalent) administered orally once daily (QD), taken approximately 30 min before the first meal of the day. The dose may be increased to 400 μg/kg/day (380 μg/kg/day free-base equivalent) QD after 1 week. Maralixibat has high solubility with an aqueous solubility >100 mg/mL but has low absorption. In the human absorption, distribution, metabolism, excretion study, approximately 72.5% and <1% of radioactivity was recovered in the feces and the urine (no parent), respectively. Less than 3% of the radioactivity was associated with the three metabolites (M1, M3, and M4). Maralixibat is not a substrate of P-glycoprotein, BCRP, OATP1B1, OATP1B3, or OATP2B1. Maralixibat is determined in vitro to be an inhibitor of OATP1B1/3 and OATP2B1 and a competitive inhibitor of CYP2C9, CYP2C19, and CYP3A. Maralixibat is a time-dependent inhibitor of CYP3A with kinact and Ki values of 0.015 min^{-1} and 5.1mM, respectively. Despite its low absorption, maralixibat has the potential to inhibit CYP3A and OATP2B1 in the intestine at clinically relevant doses. The Applicant conducted clinical drug-drug interaction studies with the sensitive CYP3A substrates simvastatin and lovastatin, and the CYP3A and OATP2B1 substrate atorvastatin in healthy adults following administration of 4.75 mg maralixibat QD; no significant interactions were observed (Studies NB4-01-02-019 and NB4-02-06-020). However, based on the proposed dosing regimen, the therapeutic dose of maralixibat could be >4.75 mg depending on body weight. The Applicant attempted to predict potential drug interactions of maralixibat with intestinal CYP3A at the proposed dosing regimen using a PBPK modeling approach.

Methods and Results

To predict potential drug interactions of maralixibat with intestinal CYP3A, an Advanced Dissolution, Absorption, and Metabolism absorption model with a mechanistic effective permeability (MechPeff) model was used in the maralixibat PBPK model. However, the ability of this model to predict the maralixibat concentrations at the interaction sites could not be verified due to insufficient data. The PBPK model of maralixibat could describe the PK of maralixibat from a single-ascending-dose study, but it failed to reproduce the PK from a multiple-ascending-dose study. In both studies, there was marked interindividual variability in maralixibat concentrations, and maralixibat was not detected at all sampling time points in all subjects. In addition, it is unclear whether the metabolites recovered in the feces were formed pre- or post-absorption. Therefore, the fraction absorbed predicted by the MechPeff model could not be verified.

To evaluate the potential drug interactions of maralixibat with a CYP3A substrate (i.e., midazolam) at doses of 5 to 45.6 mg, the reviewer used the mechanistic static model presented in Figure 7 of the FDA In Vitro Drug-Drug Interaction Guidance (FDA 2020) to predict its effect on midazolam. The results from the drug interaction studies with CYP3A substrates (Studies NB4-01-02-019 and NB4-

02-06-020) showed that maralixibat was not an inhibitor of CYP3A at the 5 mg dose. The equation in the Guidance significantly overpredicted the effect of maralixibat on CYP3A (Table 125) using the recommended inhibitor concentrations in the liver and the intestine. Therefore, the maralixibat concentrations in the liver and intestine used in the calculations were optimized such that no interaction was predicted at 5 mg. For this evaluation, midazolam was used as a probe substrate of CYP3A. The intestinal availability and fraction metabolized values of midazolam used in the prediction were 0.5 and 0.93, respectively.

The results (Table 125) showed that the study conducted at 5 mg could be reproduced only when the ka value was set to 0.1 h^{-1} and unbound inhibitor concentration in the gut and unbound plasma C_{max} or average concentration of the inhibitor were applied in the prediction. Therefore, these conditions were applied to predict the potential interaction of 26.6 or 45.6 mg maralixibat with midazolam. The 26.6 and 45.6 mg doses correspond to 380 µg/kg/day (400 µg/kg/day maralixibat chloride salt equivalent) for an individual of 70 kg and 120 kg, respectively.

Table 125. Potential Drug Interactions of Maralixibat With Midazolam by a Mechanistic Static Method

Dose (mg)	I_{liver}	I_{gut}	fa	ka	$Interaction_{gut}$	$Interaction_{liver}$	AUC_r
5	$C_{inlet,max,u}$	$I_{gut,u}$	0.03	6	1.87	2.99	5.58
	C_{max}	I_{gut}	0.03	6	1.96	1.17	2.29
	$C_{max,u}$	$I_{gut,u}$	0.03	6	1.77	1.02	1.80
	$C_{max,u}$	I_{gut}	0.03	0.1	1.40	1.02	1.42
	$C_{max,u}$	I_{gut}	0.01	0.1	1.18	1.02	1.20
	$C_{max,u}$	$I_{gut,u}$	0.03	0.1	1.06	1.02	1.08
	$C_{max,u}$	$I_{gut,u}$	0.01	0.1	1.02	1.02	1.04
	$C_{avg,u}$	$I_{gut,u}$	0.01	0.1	1.02	1.00	1.02
26.6	$C_{max,u}$	$I_{gut,u}$	0.03	0.1	1.26	1.08	1.35
	$C_{max,u}$	$I_{gut,u}$	0.01	0.1	1.10	1.08	1.19
	$C_{avg,u}$	$I_{gut,u}$	0.01	0.1	1.10	1.02	1.13
45.6	$C_{max,u}$	$I_{gut,u}$	0.03	0.1	1.37	1.13	1.54
	$C_{max,u}$	$I_{gut,u}$	0.01	0.1	1.16	1.13	1.31
	$C_{avg,u}$	$I_{gut,u}$	0.01	0.1	1.16	1.03	1.21

Source: Reviewer generated table
C_{avg} = $AUC_{0\text{-}24,ss/24}$; ka, first-order absorption rate; steady-state C_{max} or C_{avg} at various doses of maralix bat were calculated from the limited plasma PK data by assuming that maralixibat has linear PK. Unbound maximal hepatic inlet concentration ($C_{inlet,max,u}$) and Intestinal concentration (I_{gut}) of maralixibat were calculated using the equations in Figure 7 of the Guidance. $I_{gut,u}$ was calculated using the Simcyp-predicted fu,gut value of 0.096, which is similar to the unbound fraction in plasma (0.091). Fraction absorbed (fa) was 0.01 assuming that metabolites in feces were formed before absorption and was 0.03 assuming that metabolites in feces were formed after absorption. $Interaction_{gut}$ and $Interaction_{liver}$ are the gut and liver components in the equation used to calculate AUC_r in Figure 7 of the Guidance, respectively. For reference, see the applicable FDA guidance document (FDA 2020).
Abbreviations: AUC_r, area under the curve ratio; C_{max}, maximum plasma concentration; PK, pharmacokinetics

Conclusion

Maralixibat at the proposed therapeutic doses is expected to have a minimal effect on the CYP3A sensitive substrate midazolam.

14.5. Bioanalytical Methods

Quantitation of Maralixibat in Human Plasma

Concentrations of maralixibat in plasma were measured using a validated bioanalytical method. The initial method M7199012 based on liquid-liquid extraction followed by liquid chromatography with

tandem mass spectrometry was used for the initial studies in healthy subjects. Subsequently, a newer method (AS/689/00) based on protein precipitation followed by liquid chromatography with tandem mass spectrometry was developed for maralixibat in human plasma and validated in Studies 12-8929, 12-8929a, and 12-8929b. This new method (AS/689/00) was further modified to avoid signal suppression as method (AS/853/00). Table 126 summarizes the performance of the analytical method used in the pivotal study. Table 127 lists the analytical methods used in the relevant clinical studies.

Table 126. Validation Parameters of the Bioanalytical Method to Measure Maralixibat in Plasma (Method ID AS/689, Validation 12-8929)

Parameter	Description	
Materials used for standard calibration curve and concentration	Calibration standards were prepared in human plasma at 0.250, 0.500, 2.00, 5.00, 20.0, 50.0, 80.0 and 100 ng/mL	
Validated assay range	0.250 to 100 ng/mL	
Regression model and weighting	Weighted (1/×2) linear regression	
Material used for quality controls (QCs) and concentration	QC samples were prepared in human plasma at 0.250, 0.750, 15.0, 75.0, and 200 ng/mL juvenile human plasma	
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%RE) from LLOQ to ULOQ (8 runs) maralixibat (LUM001)	-2.0 to 3.4%
	Cumulative precision (%CV) from LLOQ to ULOQ (8 runs) maralixibat (LUM001)	≤5.7%
Standard calibration curve performance during accuracy and precision runs	Cumulative accuracy (%RE); 3 runs, overall) Human plasma QC levels: 0.250, 0.750, 15.0, 75.0 ng/mL (n=6/level)	-4.0 to 3.3%
	Inter-batch %CV; 3 runs, overall) Human plasma*	≤9.0%
	Juvenile plasma, QC sample performance within-batch Accuracy (%RE) Precision (%CV)	1.6 to 8.4% ≤6.5%
Selectivity and matrix effect	No interference from human plasma was observed that affected the quantification. Six individual lots of human plasma Low and High QC (n=6): Accuracy (%RE): 6.1 and 7.1% Precision (%CV): 3.5 and 4.4% Matrix factor Blank plasma from each lot was extracted and then spiked with analyte and internal standard at low and high QC levels post-extraction. The same levels were prepared in neat solvent of the same composition. Matrix factors (response ratio with matrix/ solvent) were 1.02 and 0.989 at low and high QC levels, respectively.	
Interference and specificity	Six individual lots of human plasma Blank plasma: No significant interfering peaks were observed from the analyte or internal standard.	
Extraction efficiency	Extraction recovery, maralixibat (LUM001) LQC: 108.0% HQC:106.8% Internal standard, LUM001-d4 LQC: 102.1% HQC: 96.5%	
Lipemic effect	Not assessed	
Dilution integrity, over calibration-curve QC	10-fold dilution in blank human plasma of an OCC QC sample (200 ng/mL) was demonstrated (n=6) Accuracy (%RE): 4.0 Precision (%CV): 5.1	
Bench-top/process stability	Stability in whole blood, on ice: at least 2 h and 8 min	
	Stability in human plasma at room-temperature: at least 4 h and 14 min	
	Re-injection reproducibility demonstrated for extracts held at nominally	
	4°C: 110 h and 38 min	

Parameter	Description
Freeze-thaw stability	Maralixibat (SHP625, LUM001) was shown to be stable in human plasma when stored at nominally -80°C and subjected to three freeze-thaw cycles at room temperature
Long-term storage	Maralixibat (SHP625, LUM001) was shown to be stable in human plasma:
	Nominally -80°C: at least 811 days
	Nominally -20°C: at least 71 days

Source: Summary of biopharmaceutics studies and associated analytical methods, adapted from Table 10.
Abbreviations: CV, coefficient of variation; HQC, higher quality control; LLOQ, lower limit of quantitation; LQC, lower quality control; OCC, oxygen carrying capacity; RE, random error; ULOQ, upper limit of quantitation

Table 127. Bioanalytical Methods Used to Measure Maralixibat Levels in the Clinical Studies

Study Number	Objective	Validation Report Number/Method Number	Validation Report
MRX-102	Food effect and ECG safety with to-be-marketed formulation	AS/853/00	12-8929, Amend 3
LUM001-301 (ITCH)	Safety and efficacy in ALGS	AS/689	12-8929
LUM001-302 (ITCH)	Safety and efficacy in ALGS	AS/689	12-8929
LUM001-303 (IMAGINE)	Long-term safety and efficacy in ALGS	AS/689 AS/853	12-8929, 12-8929 Amend 3
LUM001-304 (ICONIC)	Safety and efficacy in ALGS	AS/689 AS/853	12-8929, 12-8929 Amend 3
LUM001-305 (IMAGINE II)	Long-term safety and efficacy in ALGS	AS/689 AS/853	12-8929, 12-8929 Amend 3
MRX-EAP	Expanded Access Program	AS/853	12-8929 Amend 3
NB4-02-06-004 (Protocol NB4-00-02-004)	ADME and mass balance study	M7199012	M3099217
NB4-02-06-002 (Protocol NB4-99-02-002)	Single-dose safety, tolerability, PK, and PD	M7199012	M3099217
NB4-02-06-003	Multiple-dose safety, tolerability, PK, and PD	M7199012	M3099217
NB4-00-06-014 (Protocol NB4-00-02-014)	Safety, tolerability, PK, and PD	M7100010	M3000129
NB4-02-06-008 (Protocol NB4-99- 02-008)	Drug-drug interaction study with statin	M7199012	M3099217
NB4-01-06-019 (Protocol NB4-01-02-019)	Drug-drug interaction study with statin	M7100010	M3000129
NB4-02-06-020	Drug-drug interaction study with statin	M7100010	M3000129

Source: Summary of biopharmaceutics studies and associated analytical methods, adapted from Table 2, page 9.
Abbreviations: ADME, absorption, distribution, metabolism, excretion; ALGS, Alagille syndrome; EAP, expanded access program; ECG, electrocardiogram; PD, pharmacodynamic; PK pharmacokinetic

Quantitation of Bile Acids in Human Serum

Total sBA concentrations were determined using a liquid chromatography-mass spectrometry method validated in accordance with College of American Pathologists/Clinical Laboratory Improvement Amendments requirements. Concentrations of 10 conjugated and 5 unconjugated BAs (15 BAs total) were determined in serum samples using this method. Calibrators and quality control samples were prepared with known concentrations of each BA species in charcoal-stripped human serum. Serum samples were subjected to a solid-phase extraction procedure before instrumental analysis by reversed-phase-liquid chromatography combined with electrospray mass spectrometry in negative ion mode. Deuterated BAs (GCDCA-d4, CDCA-d4 and TCDCA-d4) served as internal standards for quantification. All samples from the efficacy studies in ALGS for analysis of sBAs were analyzed at (b) (4)

However, the bioanalytical method for sBA levels was not adequately validated according to the FDA Guidance for Industry Bioanalytical Method Validation (FDA 2018). As a result, the reliability of the reported sBA values cannot be assured, especially at concentrations >50 mcg/mL. Therefore, the assessment of a percentage change from baseline as the Applicant specified in the protocol is not reliable. At the same time, assessment of overall trends of sBA change using this assay can be considered feasible because the same assay was used for all the samples over time. This is an important consideration because the Applicant proposed a primary efficacy endpoint in the pivotal study LUM001-304 based on the change in fasting sBA level from Week 18 to Week 22.

The main concerns with the assay used for the measurement of sBAs in the pivotal study (LUM001-304) are discussed below. It must be noted that these concerns also apply to the other supporting efficacy studies.

Selection of QC Samples

In each analytical batch, two quality control (QC) samples of LLOQ, low, medium, and high concentrations were analyzed along with the study samples. The QC sample concentrations of bile acids were 0.1, 0.5, 1.0 and 2.5 mcg/mL in serum (batches 1 to 57). From batch 57 onwards, the calibration curve was expanded to include two more QC samples, QC 20 µg/mL and a dilution QC, QC 50 µg/mL for conjugated BAs. This is a concern as the even the expanded QC did not cover the upper range of BA concentrations (891 mcg/mL) observed in the study samples. Of note, 17 out of 29 patients had sBA > 200 µmol/L at baseline in STUDY LUM001-304which are higher than the concentrations of the QC samples used in the analysis. The highest QC sample concentrations for the glycine-conjugated and taurine-conjugated bile acids ranged from 43 – 46 µmol/L and 37 – 40 µmol/L respectively. The highest QC concentrations for the unconjugated bile acids were even lower (~ 6 – 7 mcmol/L).

As a result, majority of the serum samples for BA analysis were quantified by extrapolation above the calibration curve range. The Applicant provided additional data (Validation Memorandum Amendment 8) to support dilution of study samples to use a smaller sample volume (i.e., five-fold dilution: 10 µL sample volume versus the 50 µL originally used) which would expand the range of the QCs to 50 mcg/mL. However, even the use of a 10 µL sample does not cover the upper range of the observed bile acid concentrations. At higher concentrations there is potential for nonlinearity due to signal suppression, which could underestimate the levels of the bile acids and affect the accuracy of the estimated sBA levels.

Incurred Sample Reanalysis

The bioanalytical report indicates that Incurred Sample Reanalysis (ISR) was conducted in analytical batches 31 to 38 but not after batch 39 at the specific request of the Applicant, although no reason for this change was provided. The FDA Guidance for Industry Bioanalytical Method Validation (FDA 2018) suggests that the sample size for ISR should be at least 10% of the first 1000 samples. However, the Applicant used only 10 samples for the ISR from a total of 474 samples, which is not adequate to establish method reproducibility.

Table 128. Validation Parameters of the Bioanalytical Method for Serum Bile Acids (Method Validation Report BA-10 JUN)

Factor	Description
Materials used for standard calibration curve and concentration	Calibration standards were prepared in charcoal-stripped human serum at 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 µg/mL for each bile acid
Validated assay range	Range for each individual bile acid: 0.05 to 5 µg/mL Individual bile acids, molar units: LLOQ 0.09 to 0.13 µmol/L ULOQ 9 to 13 µmol/L
Material used for quality controls (QCs) and concentration	QC samples were prepared in charcoal stripped human serum at 0.1, 0.5, 1.0, 2.5 µg/mL for each bile acid
Regression model and weighting	Weighted (1/x) linear regression
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ 7 Cumulative accuracy (%bias) from LLOQ to ULOQ, 85 to 115% three separate occasions; 15 bile acids* 85 to 115%
Standard calibration curve performance during accuracy and precision runs	Cumulative accuracy (%bias); 15 analytes, 4 QC levels QC levels: 0.1 (LLOQ), 0.5 (low), 1.0 (Medium) and 2.5 (high) Between-run: -7.22 to 11.67 (LLOQ), -5.00 to 16.22 (low), -11.28 to 11.22 (medium), -14.24 to 1.44% (high) Within-run: -11.67 to 11.67 (LLOQ), -5.00 to 14.67 (low), -21.67 to 12.33 (medium), -31.20 to -0.27 (high) Inter-batch %CV; 15 analytes, 4 QC levels Between-run: 3.43 to 19.29 (LLOQ), 3.54 to 23.88 (low), 3.21 to 24.52 (medium), 3.88 to 23.09 (high) Within-run: 3.66 to 15.05 (LLOQ), 1.57 to 21.99 (low), 1.66 to 16.52 (medium), 1.54 to 7.22 (high)
Selectivity and matrix effect	Serum matrix lots were screened for endogenous levels of bile acids
Extraction efficiency	Recovery (bile acids): 87.8 to 103.2% (low), 87.6 to 93.8% (medium), 81.5 to 88.7% (high) Recovery (bile acid IS): 85.6 to 92.0%
Hemolysis effect	Three lots of hemolyzed blood serum was evaluated, ea. analyzed in five replicates. No significant effect on quantification was observed for total sBA or for individual bile acids.
Dilution integrity, over calibration-curve QC	Ten-fold dilution factor was evaluated
Long-term storage	Bile acids were stable in human serum (incurred samples, n=6) for at least 1 year when stored at -10°C to -25°C. Total sBA %Diff (storage vs. original): <15%
Carry over	Assessment by injecting blank plasma sample extract immediately after a high calibration standard. Carry-over was acceptable.
Freeze-Thaw stability	Analytes were stable after three freeze-thaw cycles at -10°C to -25°C
Bench-top/process stability	Processed sample stability was confirmed after 24 h when kept at 5°C

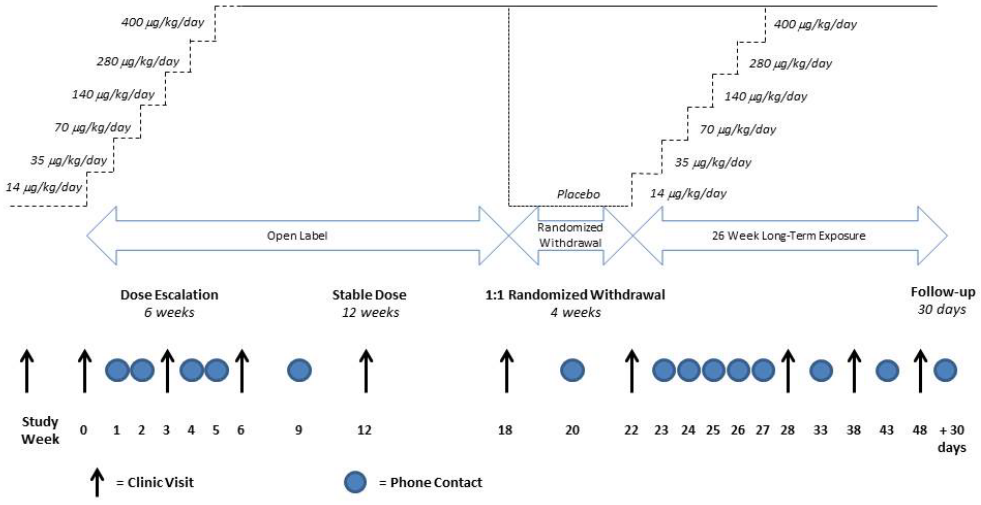
Source: Summary of biopharmaceutics studies and associated analytical methods, Adapted from Table 9, page 24.
Abbreviations: CV, coefficient of variation; LLOQ, lower limit of quantitation; sBA, serum bile acids; ULOQ, upper limit of quantitation

15. Trial Design: Additional Information and Assessment

Table 129, Table 130, and Table 131 summarize the key study design of clinical protocols for Studies LUM001-301, -302 and -304. Studies -305 and -303 were open-label extensions of -301 and -302, respectively, and are not included below. The OLE of LUM001-304 was included in study protocol 304. All studies were conducted under IND 119917, i.e., under the maralixibat development program. The data from all studies were submitted to support this new drug application (NDA). Study LUM001-304 provides the key data for efficacy and safety. Studies LUM001-301, -302, -303, and -305 provide limited supportive data for long-term safety.

Table 129. Protocol Summary, Study LUM001-304

Protocol Section	Protocol Summary
Protocol number	LUM001-304
Phase	Phase 2
Study center	This was a multicenter study, patients were enrolled at nine centers in Australia and Europe (Belgium, France, Spain, Poland, UK).
Study title	Long-term, open-label study with a double-blind, placebo-controlled, randomized drug withdrawal period of LUM001, an apical sodium-dependent bile acid transporter inhibitor (ASBTi), in patients with Alagille syndrome.
Principal investigators	Richard Schreiber, Mari del Carmen Diaz Fernandez, Loreto Hierro Llanillo, Emmanuel Jacquemin, Florence Lacaille, Alain Lachaux, Christine Rivet, Michael Stormon, Winita Hardikar, Dorota Gliwicz, Etienne Sokal, Alastair Baker
Study objectives	Objectives of the 48-week study: • Safety and tolerability of LUM001 in children with ALGS. • Effect on serum bile acid levels in children with ALGS. • Effect on biochemical markers of cholestasis. • Effect on pruritus in children with ALGS. • Evaluate the long-term effect with 48 weeks of treatment • Objectives of long-term optional follow-up treatment period (after Week 48): • Offer subjects in the LUM001-304 continued study treatment after Week 48. • Explore BID dosing regimen and higher daily dosing. • Safety and efficacy data with long term use. • Assess the level of alpha-fetoprotein (AFP). • Assess palatability.

Protocol Section	Protocol Summary
Endpoints	• Primary – Mean change from Week 18 to 22 of fasting sBA levels in subjects who previously responded to LUM001 treatment, as defined by a reduction in sBA ≥50% from baseline to Week 12 or Week 18 • Secondary – Change from Week 18 to Week 22 in: ALT, ALP, TB, DB. – Pruritus (Observer ItchRO/patient ItchRO) in subjects who previously responded to LUM001 treatment, as defined by a reduction in ItchRO scale >1 point from baseline to Week 12 or Week 18. – Change from baseline to Week 18 in: o Fasting sBA levels. o ALT, ALP, TB, and DB. o Pruritus as measured by ItchRO (ItchRO[Obs]/ItchRO[Pt]).
Study design	Multicenter, open-label, with a 4-week DB, PC, randomized withdrawal (RWD) period. Initial 18-week open-label (included a 6-week dose escalation to 400 mcg/kg), 4-week RWD, 26-week open-label after the RWD (with a 6-week dose escalation for those in the placebo arm of the RWD phase) Ten site visits (screening, Weeks 0, 3, 6, 12, 18, 22, 28, 38, 48), follow-up telephone call for those who do not roll over into the OLE Follow-up every 12 weeks for those who enroll in the OLE **Design, Study LUM001-304 (IND119917)** 
Study population	Pediatric patients with ALGS between 12 months and 18 years of age

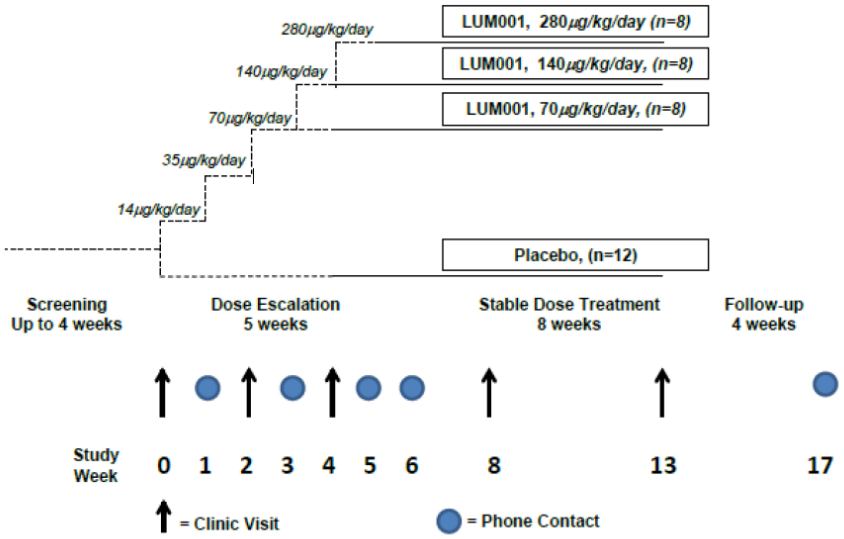
Protocol Section	Protocol Summary
Key enrollment criteria	Key inclusion criteria • Diagnosis of ALGS based on diagnostic criteria in Section II.6.2.1.2. • Significant pruritus (average daily score of >2 on the ItchRO for 2 consecutive weeks during screening). Key exclusion criteria • Chronic diarrhea requiring ongoing IVF or nutritional intervention. • Surgical interruption of the enterohepatic circulation or disease or condition that may interfere with bile salt metabolism (e.g., IBD). • Liver transplant. • Decompensated cirrhosis. • Ascites, variceal hemorrhage, encephalopathy. • INR >1.5, ALT >15× ULN, albumin <3.0 g/dL. • Bile acid or lipid binding resins within 28 days of screening. • History or presence of concomitant liver disease. • History of gallstones, kidney stones. • Pregnancy or lactation. • HIV or cancer (except for in situ carcinoma, or cancers treated at least 5 years prior to screening with no evidence of recurrence).
Sample size	N=30 (planned) N=31 (analyzed)
Dose and dosing regimen	Six-week dose escalation to the target dose of 400 mcg/kg/day Week 1 Dose: 14 mcg/kg/day QD. Week 2 Dose: 35 mcg/kg/day QD. Week 3 Dose: 70 mcg/kg/day QD. Week 4 Dose: 140 mcg/kg/day QD. Week 5 Dose: 280 mcg/kg/day QD. Week 6 Dose: 400 mcg/kg/day QD.
Duration	Forty-eight weeks with ongoing OLE until drug available commercially, participant eligible to enter another maralixibat study, or drug development discontinued.
Safety monitoring	AE, concomitant medications, vital signs, pregnancy test: All visits (screening, Weeks 0, 3, 6, 12, 18, 22, 28, 38, 48) Physical examination, skin examination: Screening, Weeks 0, 12, 18, 22, 28, 38, 48 Clinical chemistry, hematology: Weeks 0, 12, 18, 22, 28, 38, 48 Urinalysis: Weeks 0, 12, 18, 22, 28, 38, 48 INR: Screening, Week 0, 12, 18, 22, 28, 38, 48 FSVs: Weeks 0, 12, 18, 22, 28, 38, 48 Alfa-fetoprotein: Q12 weeks during OLE Office visits and safety assessments Q12 weeks during OLE AFP Q24 weeks during OLE Liver test abnormalities; repeat tests within 48-72 h in the following situations: BL ALT ≤ULN – repeat tests if ALT >5× ULN BL ALT >ULN – repeat tests if ALT >3× BL and >5× ULN BL TB 1-10 mg/dL – repeat tests if increase by ≥3 mg BL TB >10 mg/dL – repeat tests if increase by ≥5 mg
Efficacy assessments	sBA levels ItchRO (Observer/Patient) recorded twice daily ALT, ALP, TB, DB
Dose escalation	Option to increase to 400 mcg/kg BID dosing during the OLE for subjects with SBA level above normal and/or ItchRO (Obs) score ≥1.5

Protocol Section	Protocol Summary
Dose reduction	CTCAE Grade 2 or greater drug-related GI toxicity, may lower to previously tolerated dose
Treatment-interruption criteria	INR >1.5, unresponsive to vitamin K, may resume when INR falls below 1.5 or returns to BL Fasting TG >500 mg/dL, may resume when TG <300 mg/dL Treatment discontinuation for: • Diarrhea requiring treatment with IVF • ALT ≥20× ULN • TB elevations: – BL TB 1-10 mg/dL: increase of 5 mg/dL AND 2× BL – BL TB >10 mg/dL: increase of 2× BL • Pregnancy • An AE (including disease progression) that leads investigator to decide that subject should be withdrawn

Source: Adapted from Protocol Amendment 5.1, dated February 8, 2019.
Abbreviations: AE, adverse event; AFP, alpha fetoprotein; ALGS, Alagille syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BID, twice daily; BL, baseline; DB, direct bilirubin; FSV, fat-soluble vitamin; IBAT, ileal bile acid transporter; IBD, inflammatory bowel disease; INR, international normalized ratio; ItchRO, Itch Reported Outcome; ItchRO(Obs), Itch Reported Outcome (Observer); ItchRO(Pt), Itch Reported Outcome (Patient); IVF, Intravenous fluid; OLE, open-label extension; PC, placebo-controlled; sBA, serum bile acids; TB, total bilirubin; TG, triglyceride; ULN, upper limit of normal

Table 130. Protocol Summary, Study LUM001-301

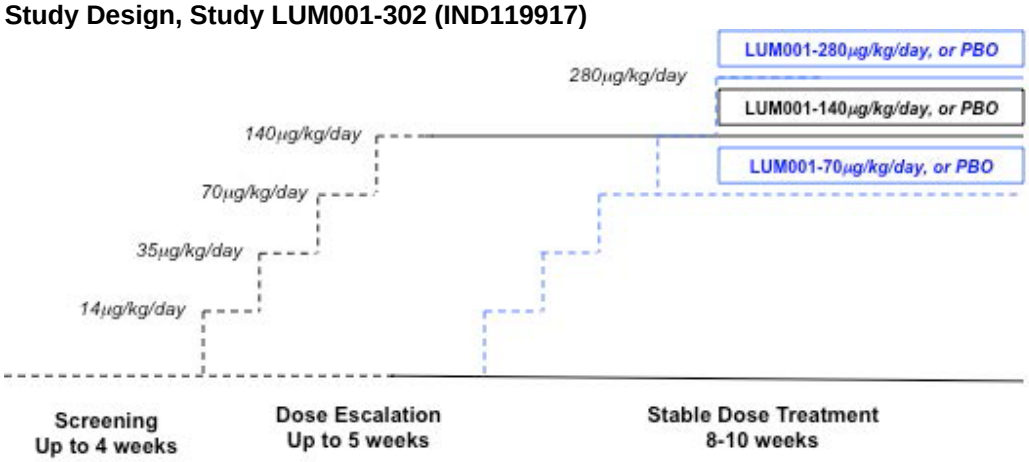
Protocol Section	Protocol Summary
Protocol number	LUM001-301
Phase	Phase 2
Study center	Multicenter study, patients were enrolled at 13 centers in the US and Canada
Study title	The evaluation of the intestinal bile acid transport (IBAT) inhibitor LUM001 in the reduction of pruritus in Alagille syndrome, a cholestatic liver disease
Principal investigators	Peter Whitington, Cara Mack, Robert Squires, Philip Rosenthal, Jean Molleston, Karen Murray, Binita Kamath, Nanda Kerkar, Danny Thomas, Kasper Wang, Saul Karpen, Stephen Guthery, Daniel Leung, Alexander Miethke, Kathleen Loomes
Study objectives	Effect on pruritus as measured by the Itch Reported Outcome (ItchRO). Safety and tolerability of LUM001. Effect on sBAs. Effect on other biochemical markers of cholestasis and liver disease.
Endpoints	Mean change from baseline to Week 13 compared with placebo • Primary – Pruritus as measured by weekly average ItchRO(Obs) at BL (7 days pretreatment) and last 7 days of treatment (Week 13). • Secondary – Fasting sBA level. – ALT, ALP, GGT, TB, and DB.

Protocol Section	Protocol Summary
Study design	Randomized, double-blind, placebo-controlled, parallel group, multicenter study with 13 weeks of treatment in children with ALGS. **Study Design, LUM001-301 (IND119917)** 
Study population	Pediatric patients with ALGS between 12 months and 18 years of age
Key enrollment criteria	Key inclusion criteria • Diagnosis of ALGS based on diagnostic criteria in Section II.6.2.1.2. • Significant pruritus (average daily score of >2 on the ItchRO for 2 consecutive weeks during screening). Key exclusion criteria • Chronic diarrhea requiring ongoing IVF or nutritional intervention • Surgical interruption of the enterohepatic circulation or disease or condition that may interfere with bile salt metabolism (e.g., IBD) • Liver transplant • Decompensated cirrhosis: • Ascites, variceal hemorrhage, encephalopathy. • INR ≥1.5, ALT >15× ULN, albumin <3.0 g/dL. • Bile acid or lipid binding resins within 28 days of screening. • History or presence of concomitant liver disease. • Pregnancy or lactation. • HIV or cancer (except for in situ carcinoma, or cancers treated at least 5 years prior to screening with no evidence of recurrence).
Sample size	N=36 (planned) N=37 (analyzed)

Protocol Section	Protocol Summary
Dose and dosing regimen	70 mcg/kg/day (n=8) 140 mcg/kg/day (n=8) 280 mcg/kg/day (n=8) Placebo (n=12) Dose escalation over 3-5 weeks, depending on the goal dose: Week 1 Dose: 14 mcg/kg/day QD. Week 2 Dose: 35 mcg/kg/day QD. Week 3 Dose: 70 mcg/kg/day QD. Week 4 Dose: 140 mcg/kg/day QD. Week 5 Dose: 280 mcg/kg/day QD.
Duration	Thirteen weeks, followed by optional ongoing OLE (Study LUM001-305) until drug available commercially, participant eligible to enter another maralixibat study, or drug development discontinued.
Safety monitoring	AE, concomitant medications, vital signs, pregnancy test: All visits (screening, Weeks 0, 2, 4, 8, 13) Physical examination, skin examination: Screening, Weeks 0, 2, 4, 8, 13 Clinical chemistry, hematology: Screening, Weeks 0, 2, 4, 8, 13 Urinalysis: Screening, Weeks 0, 2, 4, 8, 13 INR: Screening, Weeks 0, 2, 4, 8, 13 FSVs: Weeks 0, 8,13 PK assessments: Weeks 0, 2, 4, 8, 13 Liver test abnormalities, repeat tests within 14 days of initial elevation: BL ALT ≤ ULN – repeat tests if ALT >5× ULN. BL ALT >ULN – repeat tests if ALT >3× BL and >5× ULN. BL TB 1-10 mg/dL – repeat tests if increase by ≥3 mg. BL TB >10 mg/dL – repeat tests if increase by ≥5 mg.
Efficacy assessments	ItchRO(Obs) recorded twice daily (weekly average as endpoint). Fasting sBAs. ALT, ALP, GGT, TB, DB.
Dose reduction	CTCAE Grade 2 or greater drug-related GI toxicity – may lower to previously tolerated dose
Treatment-interruption criteria	• INR >1.5, unresponsive to vitamin K, may resume when INR falls below 1.5 or returns to baseline level • Fasting TG >500 mg/dL, may resume when TG <300 mg/dL • Treatment discontinuation for: – Diarrhea requiring treatment with IVF. – ALT ≥20× ULN. • TB elevations: – BL TB 1-10 mg/dL: increase of 5 mg/dL AND 2× BL. – BL TB >10 mg/dL: increase of 2× BL. • Pregnancy. • An AE (including disease progression) that leads investigator to decide that subject should be withdrawn.

Source: Adapted from Protocol Amendment 3, Dated February 11, 2015.
Abbreviations: AE, adverse event; ALGS, Alagille syndrome; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline; DB, direct bilirubin; FSV, fat-soluble vitamin; GGT, gamma glutamyltransferase; GI, gastrointestinal; IBAT, ileal bile acid transporter; IBD, inflammatory bowel disease; INR, international normalized ratio; ItchRO, Itch Reported Outcome; ItchRO(Obs), Itch Reported Outcome (Observer); ItchRO(Pt), Itch ReportedOutcome (Patient); IVF, intravenous fluid; OLE, open-label extension; PK, pharmacokinetic; QD, once daily; sBA, serum bile acids; TB, total bilirubin; TG, triglyceride; ULN, upper limit of normal

Table 131. Protocol Summary, Study LUM001-302

Protocol Section	Protocol Summary
Protocol number	LUM001-302
Phase	Phase 2
Study center	This was a multicenter study; patients were enrolled at three centers in the UK
Study title	A randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of LUM001, an apical sodium-dependent bile acid transporter inhibitor (ASBTi), in the treatment of cholestatic liver disease in pediatric patients with Alagille syndrome
Principal investigators	Alastair Baker, Deirdre Kelly, Patricia McClean, Richard Thompson
Study objectives	• Safety and tolerability. • Effect on sBAs. • Effect on liver enzymes. • Effect on pruritus associated with ALGS. • Effect on other biochemical markers associated with ALGS.
Endpoints	Mean change from baseline to Week 13 compared with placebo. • Primary: – Fasting sBA level. • Secondary: – Pruritus as measured by weekly average ItchRO (ItchRO[Obs]/ItchRO[Pt]) at BL (7 days pretreatment) and last 7 days of treatment (Week 13). – ALT, AST, ALP
Study design	Cohort A: 140 mcg/kg/day LUM001 (n=6), or placebo (n=3). Cohort B: 70 or 280 mcg/kg/day LUM001 (n=6), or placebo (n=3). The dose level for Cohort B (70 mcg/kg/day or 280 µg/kg/day) will be selected based on tolerability results from Cohort A (140 mcg/kg/day) at Week 4. **Study Design, Study LUM001-302 (IND119917)** 
Study population	Pediatric patients with ALGS between 12 months and 18 years of age

Protocol Section	Protocol Summary
Key enrollment criteria	• Key inclusion criteria – Diagnosis of ALGS based on diagnostic criteria in Section II.6.2.1.2. – Significant pruritus (average daily score of >2 on the ItchRO for 2 consecutive weeks during screening). • Key exclusion criteria – Chronic diarrhea requiring ongoing IVF or nutritional intervention. – Surgical interruption of the enterohepatic circulation or disease or condition that may interfere with bile salt metabolism (e.g., IBD). – Liver transplant. – Decompensated cirrhosis: – Ascites, variceal hemorrhage, encephalopathy. – INR ≥1.5, ALT >15× ULN, albumin <3.0 g/dL. – Bile acid or lipid binding resins within 28 days of screening. – History or presence of concomitant liver disease. – Pregnancy or lactation. – HIV or cancer (except for in situ carcinoma, or cancers treated at least 5 years prior to screening with no evidence of recurrence).
Sample size	N=18 (planned) N=20 (analyzed)
Dose and dosing regimen	140 mcg/kg/day (n=6) 280 mcg/kg/day (n=6) Placebo (n=6) Dose escalation over 3-5 weeks, depending on the goal dose: • Week 1 dose: 14 mcg/kg/day QD. • Week 2 dose: 35 mcg/kg/day QD. • Week 3 dose: 70 mcg/kg/day QD. • Week 4 dose: 140 mcg/kg/day QD. • Week 5 dose: 280 mcg/kg/day QD.
Duration	Thirteen weeks, followed by optional ongoing OLE (Study LUM001-303) until drug available commercially, participant eligible to enter another maralixibat study, or drug development discontinued.
Safety monitoring	AE, concomitant medications, vital signs, pregnancy test: all visits (screening, Weeks 0, 2, 5, 9, 13) Physical examination, skin examination: Screening, Weeks 0, 2, 5, 9, 13 Clinical chemistry, hematology: Screening, Weeks 0, 2, 5, 9, 13 Urinalysis: Screening, Weeks 0, 2, 5, 9, 13 INR: Screening, Weeks 0, 2, 5, 9, 13 FSVs: Weeks 0, 9,13 PK assessments: Weeks 0, 5, 13 Liver test abnormalities, repeat tests within 3 days of initial elevation: BL ALT or AST ≤ULN – repeat tests if ALT or AST >5× ULN. BL ALT or AST >ULN – repeat tests if ALT or AST >3× BL. If ALT or AST >2× BL AND TB >2× BL.
Efficacy assessments	ItchRO(Obs)/ItchRO(Pt) recorded twice daily (weekly average as endpoint). Fasting sBAs. ALT, AST, ALP.
Dose reduction	CTCAE Grade 2 or greater drug-related GI toxicity, may lower to previously tolerated dose

Protocol Section	Protocol Summary
Treatment-interruption criteria	INR >1.5, unresponsive to vitamin K, may resume when INR falls below 1.5 or returns to baseline level Fasting TG >500 mg/dL, may resume when TG <300 mg/dL Treatment discontinuation for: Pregnancy. An AE (including disease progression) that leads investigator to decide that subject should be withdrawn. Liver test abnormalities, in the absence of an alternative explanation: IF BL ALT or AST ≤ULN to 2.5× ULN, discontinue if ALT or AST ≥8× ULN. IF BL ALT or AST >2.5× ULN to 5.0× ULN, discontinue if ALT or AST ≥10× ULN. IF BL ALT or AST >5.0× ULN to 15.0× ULN, discontinue if ALT or AST ≥10× ULN **and** 2× BL. For all subjects, discontinue if: A 2× increase over ALT or AST BL **and** a 2× increase in TB BL **or** INR >1.5 A 2× increase over ALT or AST BL and the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or concomitant eosinophilia (>5%).

Source: Adapted from Protocol Amendment 4, Dated February 25, 2014.
Abbreviations: AE, adverse event; ALGS, Alagille syndrome; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline; DB, direct bilirubin; FSV, fat-soluble vitamin; GGT, gamma glutamyltransferase; GI, gastrointestinal; IBAT, ileal bile acid transporter; IBD, inflammatory bowel disease; INR, international normalized ratio; ItchRO, Itch Reported Outcome; ItchRO(Obs), Itch Reported Outcome (Observer); ItchRO(Pt), Itch Reported Outcome (Patient); IVF, intravenous fluid; OLE, open-label extension; PK, pharmacokinetic; QD, once daily; sBA, serum bile acids; TB, total bilirubin; TG, triglyceride; ULN, upper limit of normal

16. Efficacy: Additional Information and Assessment

16.1. Clinical Outcome Assessments

16.1.1. Qualitative Descriptions of COAs Used in the Study

Itch-Related Instruments

ItchRO(Obs): Observer-Reported Outcome Measure of Itch-Related Symptom Severity

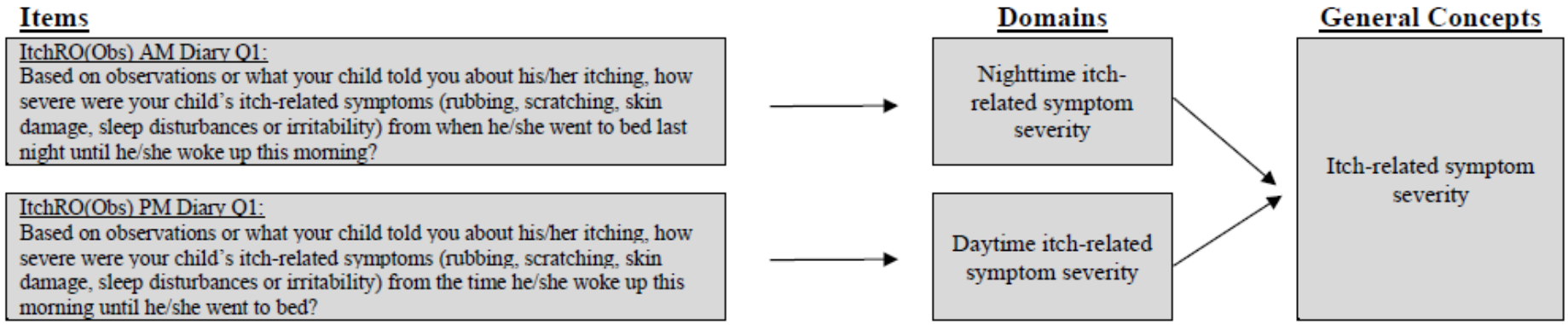
The ItchRO(Obs) item 1 is designed to assess itch-related symptom severity on a 5-point verbal rating scale that includes the following response options: “Not observed”, “Mild”, “Moderate”, “Severe”, and “Very Severe.” There are two versions of the instrument, AM (assesses itch-related symptoms during nighttime) and PM (assesses itch-related symptoms during daytime). The recall period is over the previous half day (e.g., since he/she went to bed last night until he/she woke up this morning for AM, and from the time he/she woke up this morning until he/she went to bed for PM). See Figure 31.

Itch Reported Outcome (Patient): Patient-Reported Outcome Measure of Itching Severity

The Itch Reported Outcome (Patient) item 1 is designed to assess itch intensity on a 5-point verbal rating scale that includes the following response options: “I didn’t feel itchy”, “I felt a little bit itchy”, “I felt pretty itchy”, “I felt very itchy”, and “I felt very, very itchy.” There are two versions of the instrument, AM (assesses itch during nighttime) and PM (assesses itch during daytime). The

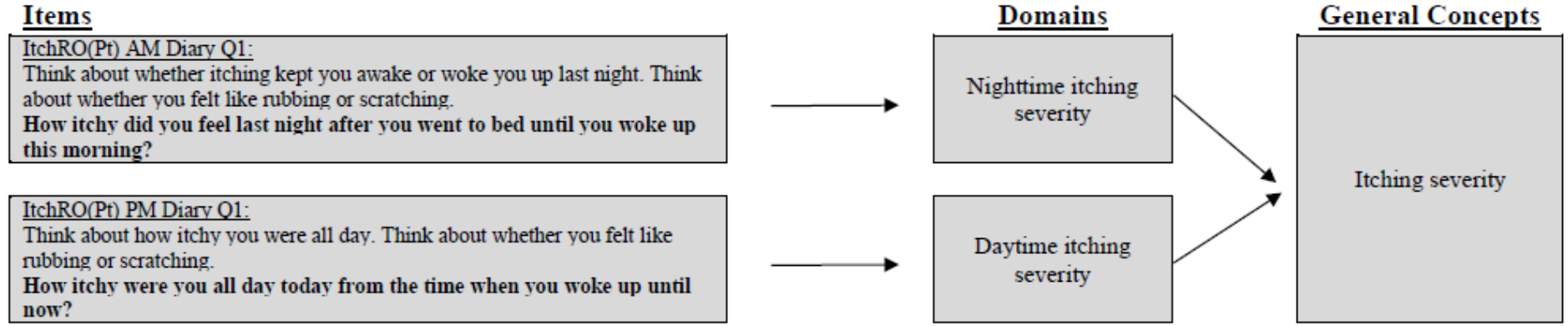
recall period is over the previous half day (e.g., after you went to bed until you woke up this morning for AM, and from the time when you woke up until now for PM). See Figure 32.

Figure 31. Conceptual Framework for ItchRO(Obs)



Source: Development of the Pediatric ItchRO Dossier Figure 1, page 19.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer)

Figure 32. Conceptual Framework for ItchRO(Pt)



Source: Development of the Pediatric ItchRO Dossier Figure 2, page 20.
Abbreviations: ItchRO(Pt), Itch Reported Outcome (Patient)

Anchor Scales

Caregiver Impression of Change in Itch

The Caregiver Impression of Change is designed to capture the caregiver's assessment of change in the patient's itch-related symptoms after various points of study drug treatment compared to that prior to the start of treatment. The Caregiver Impression of Change uses a 7-point scale with the following response options: 1=Much better, 2 = Better, 3 = A little better, 4 = No change, 5 = A little worse, 6 = Worse, 7 = Much worse.

Clinician Scratch Scale

The Clinician Scratch Scale is a 5-point scale to be used by clinicians to rate the patient's itch severity, based on the clinicians' observations of scratching (e.g., damage to the skin as a result of scratching). The scale includes the following response options: 0 "None", 1 "Rubbing of mild scratching when undistracted", 2 "Active scratching without evident skin abrasions," 3 "Abrasion evident", and 4 "Cutaneous mutilation, hemorrhage and scarring evident." The Clinician Scratch Scale assesses the clinician's observation of scratching at the time of administration with essentially no past recall period.

16.1.2. Timing of ItchRO(Obs) Morning and Evening eDiary Entries

Although the morning and evening ItchRO(Obs) electronic diaries were designed to be completed independently twice daily (once in the morning and once in the evening, respectively), caregivers were able to complete both the morning and evening ItchRO(Obs) once within a 24 h period when circumstances prevented them from completing the assessments twice daily. Because completing the morning and evening diaries at one time could negatively affect the integrity of the study data, the FDA examined the interval between caregivers' ItchRO(Obs) Morning and Evening diary entries.

During the RWD period (i.e., Week 18 to Week 22) there were three cases of both morning and evening diaries being completed within less than 6 h of each other, from a total of two caregivers. The Patient-Focused Statistical Support team concluded that the small number of affected cases should have minimum impacts in the overall study data quality during the RWD period.

16.1.3. Comparability of Morning and Evening ItchRO(Obs) Scores and Stability of Scores Over Time

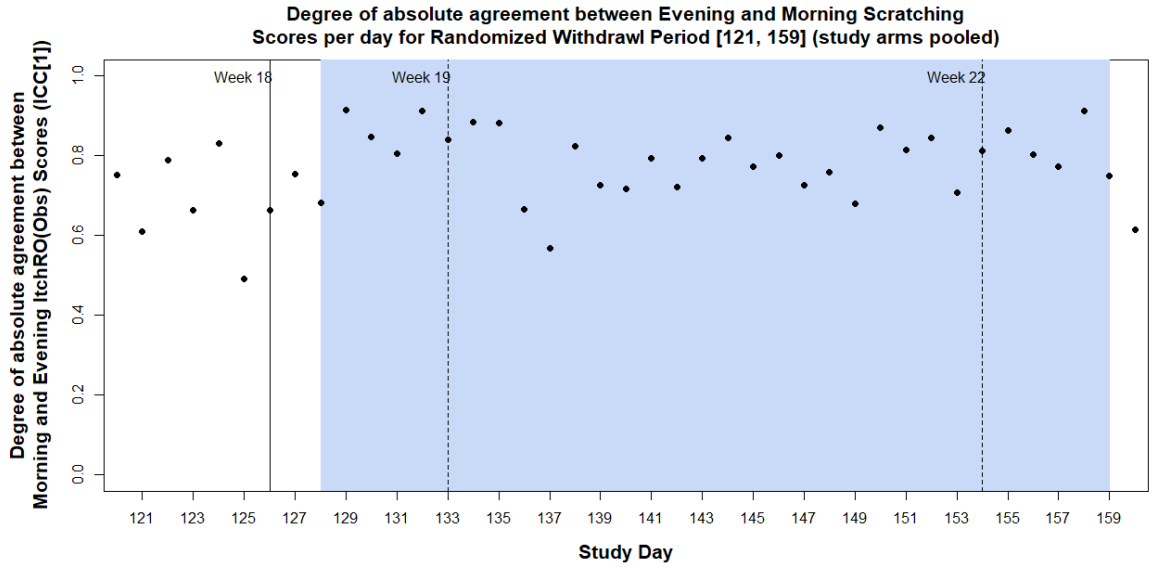
Daily ItchRO(Obs) Scores

The comparability of morning and evening ItchRO(Obs) item 1 scores (hereafter referred to as ItchRO(Obs) scores) within a given Study Day and the stability of ItchRO(Obs) scores over time were evaluated to inform the construction of an appropriate daily ItchRO(Obs) score. A scatterplot of the intraclass correlation between morning and evening ItchRO(Obs) scores on a given study day during the randomized withdrawal period (i.e., Week 18 to Week 22) was

visually examined. For each study day, the intraclass correlation coefficient (ICC(1)) based on the absolute agreement between morning and evening ItchRO(Obs) scores that day was computed (McGraw and Wong 1996) based on a mixed model with no interaction term with ItchRO(Obs) score as the response variable, patients included as a random effect, and item (i.e., the morning and evening observer-reported outcome measures of pruritus) included as a fixed effect.

Figure 33 shows the degree of absolute agreement between morning and evening ItchRO(Obs) scores within a given day for the RWD period, which is shaded in blue. There is moderate agreement between the morning and evening ItchRO(Obs) scores on a given study day and a high level of day-to-day variability in the degree of absolute agreement between morning and evening ItchRO(Obs) scores. These findings indicated that it may be most informative to look at a patient's *worst pruritus severity* within a day to accommodate differences between morning and evening pruritus severity within a given day, and also capture the patient's worst experience that day. This finding provided further support for the endpoint "change from Week 18 (pre-randomization) to Week 22 in the weekly average of the worst daily ItchRO(Obs) scores (the largest of the morning and evening scores each day)," as specified in the protocol (see Section II.6.2.1.3).

Figure 33. Degree of Absolute Agreement Between Morning and Evening ItchRO(Obs) Scores per Day for the Randomized Withdrawal Period

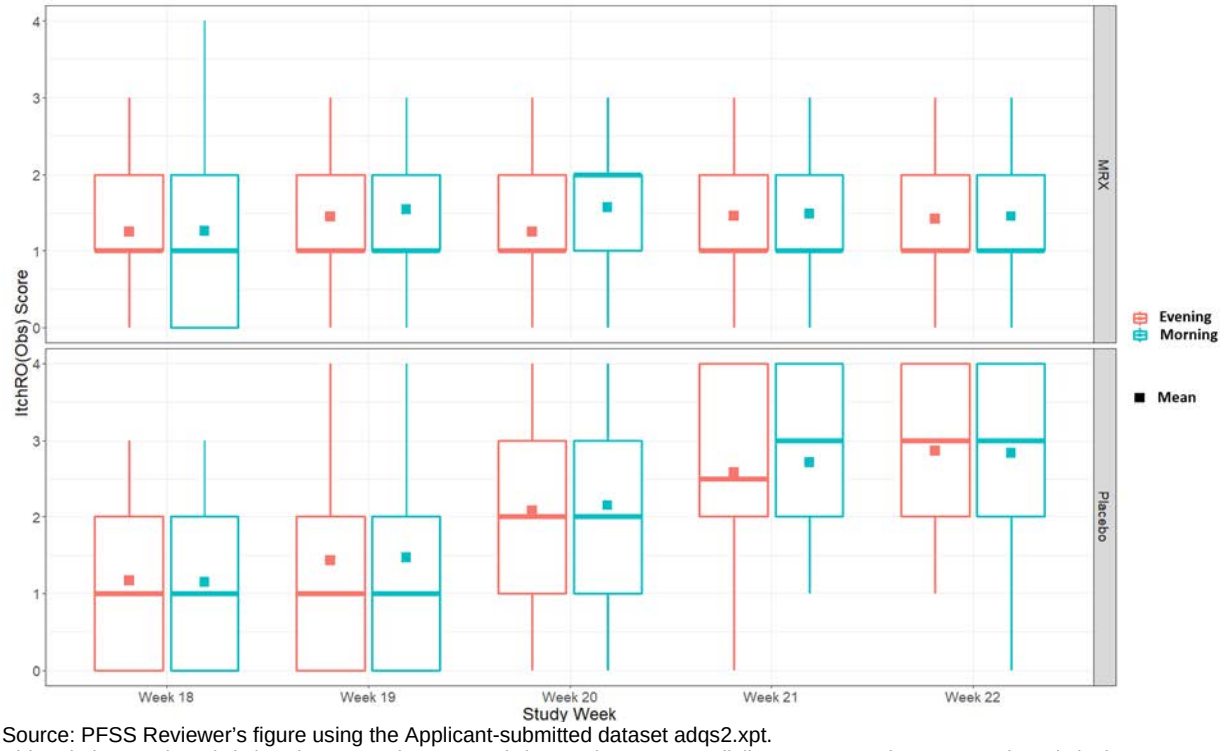


Source: PFSS Reviewer's figure using the Applicant-submitted dataset adqs2.xpt.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); PFSS, Patient-Focused Statistical Support

Weekly ItchRO(Obs) Scores

The comparability of weekly average morning and weekly average evening ItchRO(Obs) scores during the RWD period was examined by treatment arm. Figure 34 shows that the distribution of weekly average morning ItchRO(Obs) scores (blue) was comparable to that of weekly average evening ItchRO(Obs) scores (coral) within each treatment arm. The top panel shows that the weekly average morning and weekly average evening scores tended to be similar in both means and medians for the treatment group. The bottom panel shows that weekly average morning and weekly average evening scores tend to increase by week in the placebo group.

Figure 34. Distribution of Weekly Average Morning and Evening ItchRO(Obs) Scores During the Randomized Withdrawal Period by Treatment Arm

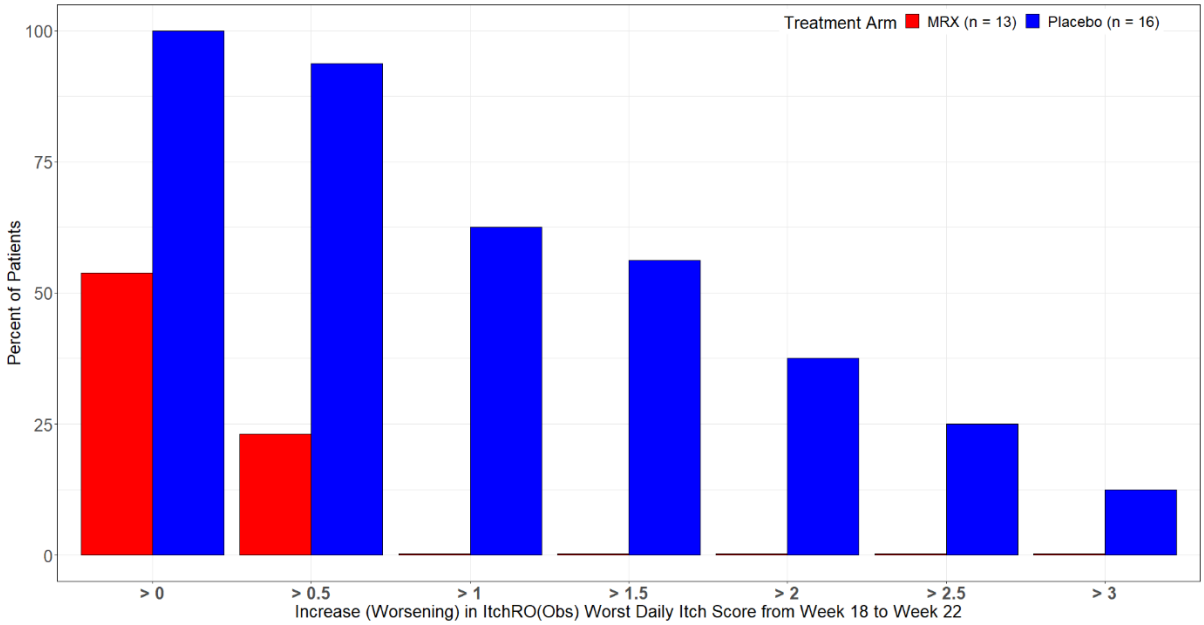


Source: PFSS Reviewer's figure using the Applicant-submitted dataset adqs2.xpt.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PFSS, Patient-Focused Statistical Support

16.1.4. Interpretation of Efficacy: Randomized Withdrawal Period

As discussed in Section II.6.3.5, the FDA concluded that anchor-based analyses of clinically meaningful within-patient change in pruritus were not needed to interpret the treatment effect in Study LUM001-304. The FDA performed an exploratory descriptive analysis to examine the difference in the change in worst daily ItchRO(Obs) score between patients who continued on treatment (maralixibat group) and those who were withdrawn from treatment (placebo group) during the RWD period. Figure 35 displays the percentages of patients who experienced various levels of worsening (i.e., any numerical increase in pruritus scores from Week 18) in pruritus during the RWD period. Approximately 29% of patients who remained on treatment experienced no change compared to 0% of patients who were withdrawn from treatment. Approximately 54% of patients who remained on treatment during the RWD period experienced worsening compared to 100% of patients who were withdrawn from the treatment. No patients in the treatment group experienced a worsening in the worst daily ItchRO(Obs) score of greater than 1 point, whereas approximately 62% of patients in the placebo group experienced a worsening of 1 point or greater. In addition, although not shown in Figure 35, approximately 29% of patients who remained on treatment experienced no change compared to 0% of patients who were withdrawn from treatment. These differences in the change in worst daily ItchRO(Obs) score during the RWD period support the conclusion that the treatment effect is meaningful for patients.

Figure 35. Percentage of Patients Who Experienced Various Levels of Increase (Worsening) in Worst Daily ItchRO(Obs) Scores During the Randomized Withdrawal Period



Source: PFSS Reviewer's figure using the Applicant-submitted dataset adqs2.xpt.
Worsening is defined as any numerical increase in pruritus scores from Week 18.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PFSS, Patient-Focused Statistical Support

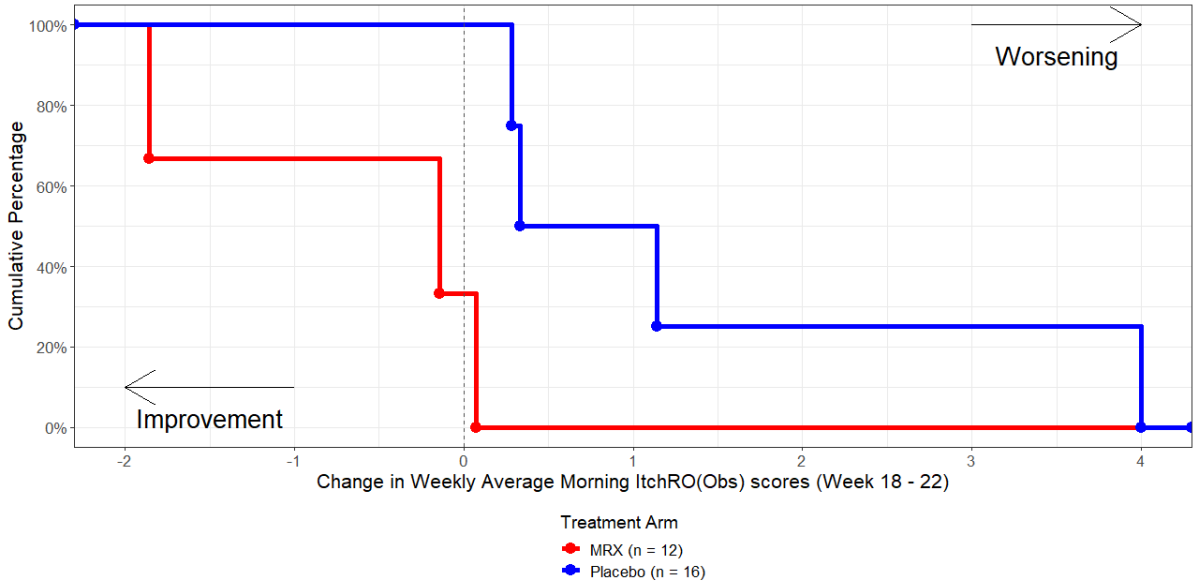
The FDA also conducted post hoc analyses of empirical cumulative distribution function curves of within-patient changes in pruritus scores from baseline by treatment arm for the following pruritus endpoints of interest (see Section II.6.3.5).

Change from Week 18 (pre-randomization) to Week 22 in the weekly average of the:

- Worst daily ItchRO(Obs) scores.
- Daily average of the morning and evening ItchRO(Obs) scores.
- Morning ItchRO(Obs) scores.
- Evening ItchRO(Obs) scores.

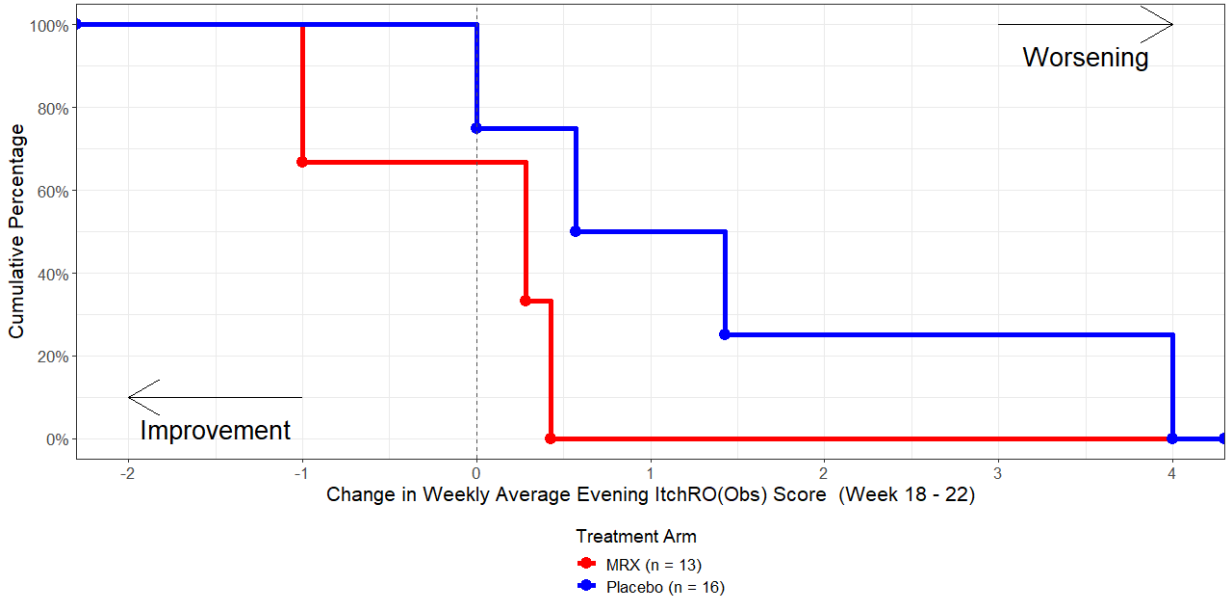
Discussion of the empirical cumulative distribution function curves by treatment arm for the endpoint change in weekly average of worst daily ItchRO(Obs) scores are presented in Section II.6.3.5. Figure 36, Figure 37, and Figure 38 present the empirical cumulative distribution function curves for the other three pruritus endpoints.

Figure 36. eCDF Curves by Treatment Arm for Change in Weekly Average Morning ItchRO(Obs) Score During the Randomized Withdrawal Period



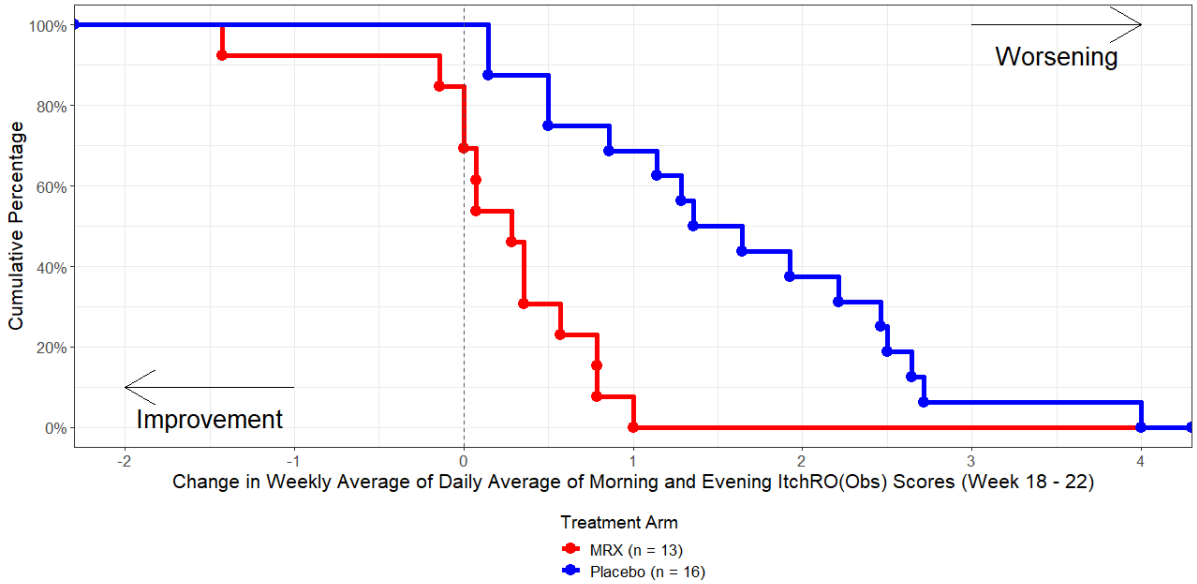
Source: PFSS Reviewer's figure using the Applicant-submitted dataset adqs2.xpt.
Abbreviations: eCDF, empirical cumulative distribution function; ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PFSS, Patient-Focused Statistical Support

Figure 37. eCDF Curves by Treatment Arm for Change in Weekly Average Evening ItchRO(Obs) Score During the Randomized Withdrawal Period



Source: PFSS Reviewer's figure using the Applicant-submitted dataset adqs2.xpt.
Abbreviations: eCDF, empirical cumulative distribution function; ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PFSS, Patient-Focused Statistical Support

Figure 38. eCDF Curves by Treatment Arm for Change in Weekly Average of Daily Average of Morning and Evening ItchRO(Obs) Scores During the Randomized Withdrawal Period



Source: PFSS Reviewer's figure using the Applicant-submitted dataset adqs2.xpt.
Abbreviations: eCDF, empirical cumulative distribution function; ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat; PFSS, Patient-Focused Statistical Support

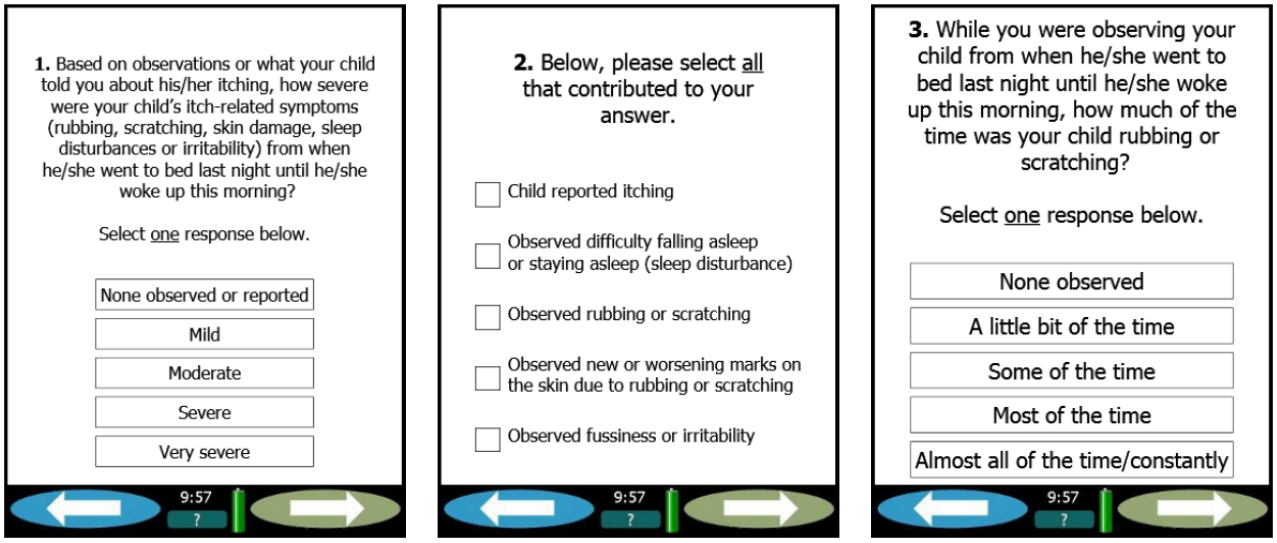
16.1.5. COA Appendices

Table 132. Documents Submitted Under NDA 214662 Reviewed in Quantitative COA-Focused Quantitative Evaluation

Document	Date Received
ItchRO Dossier: Development of the Pediatric ItchRO: A Clinical Outcome Assessment Tool for the Evaluation of Itching in Pediatric Cholestatic Liver Disease • Report on the Psychometric Validation of the ItchRO Diaries in Pediatric Cholestatic Liver Disease version 3.0 (dated April 22, 2016) • Statistical Analysis Plan (SAP) for the Validation of the ItchRO Diaries in Pediatric Cholestatic Liver Disease version 7.0 (dated April 27, 2015) • Development and Psychometric Evaluation of the Itch Report Outcome (ItchRO) – March 6, 2019 • Final Statistical Analysis Plan, version 1.0, dated March 17, 2020 • Supplementary Psychometric Evaluation of the ItchRO in Participants with Alagille Syndrome Report (final version 1.1, October 7, 2020) • Supplementary Psychometric Evaluation of the ItchRO in Participants with Alagille Syndrome Report (final version 1.1; October 7, 2020) • Supplementary Psychometric Evaluation of the ItchRO in Participants with Alagille Syndrome Report Appendices – Appendix C: Itch Reported Outcome Measure screen shots – Appendix D: Validation Analysis Plan for the Supplementary Psychometric Evaluation of the ItchRO in Participants with Alagille Syndrome (final version 2.0; September 20, 2019) – Appendix F: Patient Profile Plots – Appendix H: Empirical Cumulative Distribution Function Figures • Final Statistical Analysis Plan, version 1.0, dated March 17, 2020	November 12, 2020
LUM001-304 Final CSR – Clinical Study Report Body (final version 2.0; November 11, 2020)	May 21, 2021
Clinical Information Amendment submitted in response to an FDA Information Request (dated March 26, 2021)	March 31, 2021

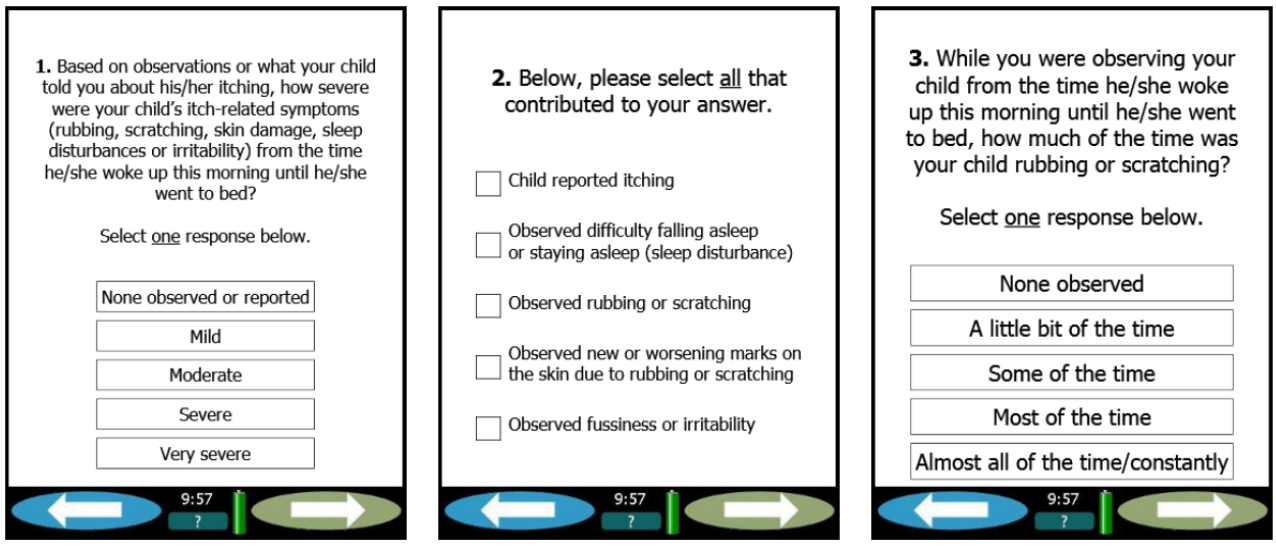
Source: PFSS Reviewer.
Abbreviations: COA, Clinical Outcome Assessment; FDA, Food and Drug Administration; PFSS, Patient-Focused Statistical Support

Figure 39. ItchRO (Obs) Daily Morning (AM) eDiary, Study LUM001-304



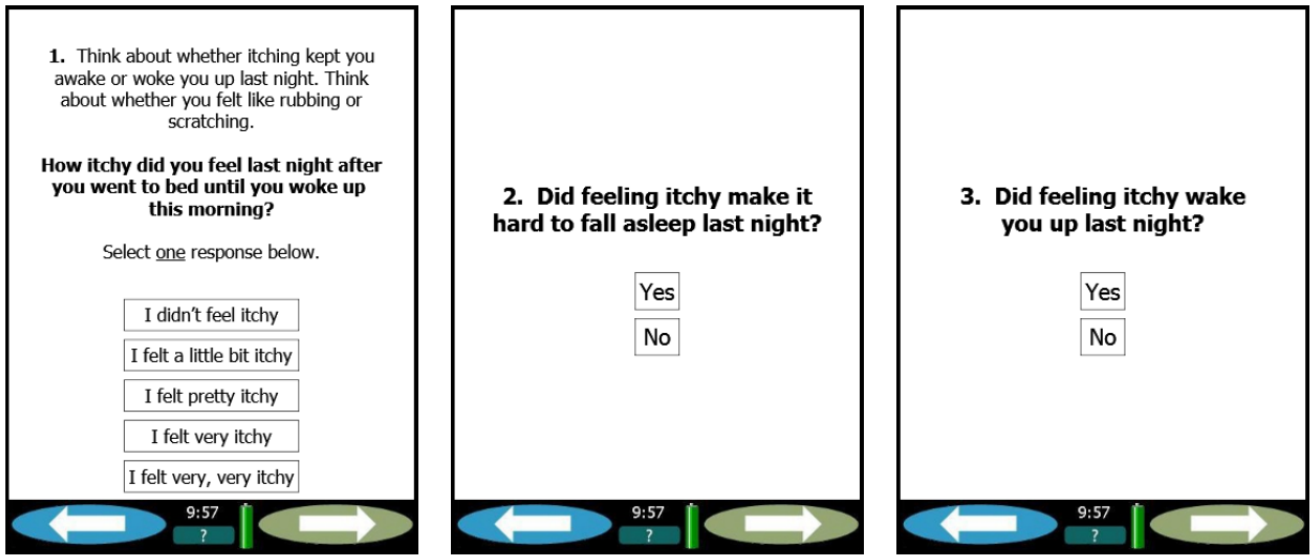
Source: ItchRO Dossier Section 2.1.1, page 10/2939.
Abbreviations: ItchRO (Obs), Itch Reported Outcome (Observer)

Figure 40. ItchRO (Obs) Daily Evening (PM) eDiary, Study LUM001-304



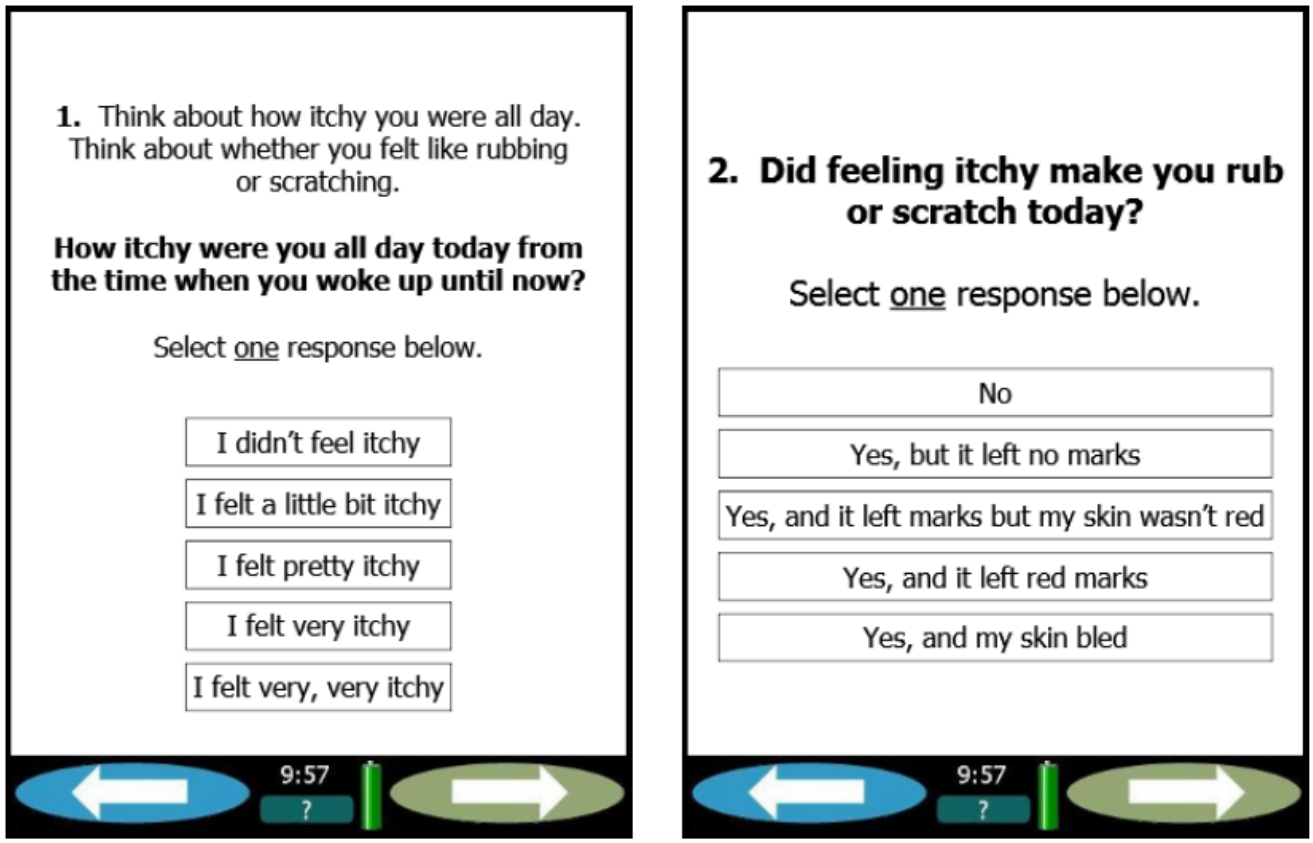
Source: ItchRO Dossier; Section 2.1.2, page 11/2939.
Abbreviations: ItchRO (Obs), Itch Reported Outcome (Observer)

Figure 41. ItchRO (Pt) Daily Morning (AM) eDiary, Study LUM001-304



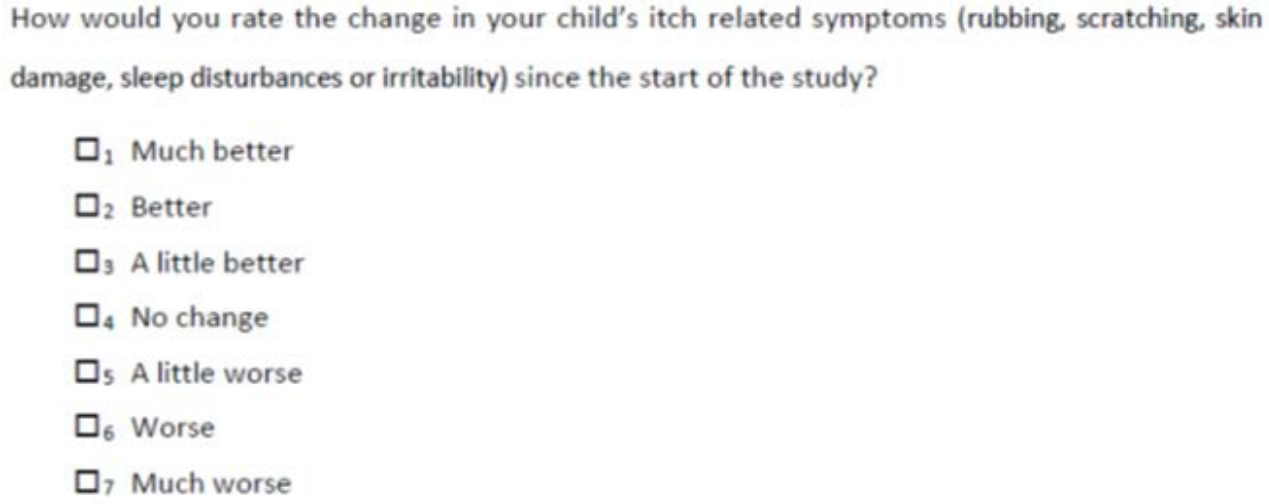
Source: ItchRO Dossier; Section 2.2.1, page 11/2939.
Abbreviations: ItchRO (Pt), Itch Reported Outcome (Patient)

Figure 42. ItchRO (Pt) Daily Evening (PM) eDiary, Study LUM001-304



Source: ItchRO Dossier; Section 2.2.2, page 12/2939.
Abbreviations: ItchRO (Pt), Itch Reported Outcome (Patient)

Figure 43. Caregiver Impression of Change in Itch, Study LUM001-304



Source: Development of the Pediatric ItchRO Dossier, Appendix K of the study manual.

Figure 44. Clinician Scratch Scale, Study LUM001-304

The clinician will rate the subject's pruritus, as evidenced by scratching, according to the following scale:

Score	Description
0	None
1	Rubbing or mild scratching when undistracted
2	Active scratching without evident skin abrasions
3	Abrasion evident
4	Cutaneous mutilation, hemorrhage and scarring evident

Source: Development of the Pediatric ItchRO Dossier, Appendix C, 16.5.

16.2. Timeline of Protocol Amendments and Study Events

Changes implemented in the protocol amendments are quoted from the Applicant's summary in Clinical Study Report LUM001-304.

- **March 2014** – Original protocol.
- **March 2015** – Protocol Amendment 1: "add exclusion criteria of history or presence of gallstones or kidney stones and known hypersensitivity to maralixibat or any of its components."
- **May 2015** – Protocol Amendment 2: "add exclusion of participants weighing over 50 kg at screening, change maximum daily dose from 30 mg/day to 20 mg/day, responder definition corrected from Week 18 to Week 12, and language added to address the randomization and statistical management of data generated from siblings enrolled in the study and to describe a planned extension study."
- **November 2015** – Protocol Amendment 3: "add an optional follow-up treatment period (after Week 48) that allowed eligible participants treated in Study LUM001-304 to continue on treatment after Week 48 until the first of the following occurred: (i) up to

52 weeks of additional treatment (Week 100), or (ii) in the event that a new study opened to enrollment; add an objective to obtain safety and efficacy data in participants treated long term on maralixibat, including genotyping characteristics; add NOTCH2 to list of laboratory analytes."

- **April 2016** – Last patient Week 22 study visit.
- **October 2016** – Last patient Week 48 study visit.
- **March 2017** – Protocol Amendment 4 allows planned unblinded interim analysis after all subjects completed Week 48 or discontinued prior to Week 48. "Allow continued participation in the optional follow-up treatment period, beyond what had been described in Protocol Amendment 3; clarify that study treatment in the optional follow-up treatment period could continue until the first of the following occurred: 1) the participants were eligible to enter another maralixibat study, 2) maralixibat was available commercially, or 3) the sponsor stopped the program or development in this indication; clarify that eligible participants who had previously discontinued from the study could re-enter and receive study treatment in the optional follow-up treatment period; and describe objectives and assessments of the optional follow-up treatment period, including the following: exploration of a BID dosing regimen and higher daily dosing of maralixibat; assessment of alfafeto protein levels, a marker of hepatocellular carcinoma; assessment of the palatability of the maralixibat formulation in all participants, by-proxy in participants <4 years old and by-participant questionnaire in children ≥4 years old; and update the contraceptive requirements."
- **November 2017** – Protocol Amendment 5: "change the study design going forward to an OL study beyond what had been described in Protocol Amendment 4; to add details regarding interim analyses up to Week 48 and unblinding of the study; and to add clinician xanthoma scale to Schedule of Procedures."
- **January 2018** – Interim analysis unblinding form was completed to allow only the Applicant Global Development Lead to be unblinded in order to review the raw data to support decision making on the continuation of the development program in ALGS.
- **February 2018** – Week 48 Statistical "Interim Analysis" Plan was finalized.
- **March 2018** – Database freeze, study team unblinded.
- **June 2018** – Analyses conducted using up to Week 48 data.
- **February 2019** – Protocol Amendment 5.1: "reflect the change of sponsorship from Lumena Pharmaceuticals LLC to Mirum Pharmaceuticals, Inc, and to document the change in medical monitor."
- **March 2019** – Meeting package submitted to FDA with study results.
- **March 2020** – SAP finalized.

16.3. Additional Efficacy Information

Missing Data

Table 133 presents the amount of missing data for the weekly averages of ItchRO(Obs) scratching severity scores calculated using different summaries of the daily data: worst of the morning and evening scores, average of the morning and evening scores, morning score, evening score. A weekly average is considered missing if fewer than four of the seven daily ItchRO(Obs) scores are reported for the 7-day period. As only one score (morning or evening) on a given day

is needed to calculate the worst and average daily summaries, these summary measures had the same missing data pattern and are presented together.

Table 133. Missing Data for Weekly Averages of ItchRO(Obs) Scratching Severity Scores at Key Timepoints, Study LUM001-304

	Worst and Average Daily Scores		Daily Morning Score		Daily Evening Score	
ItchRO(Obs) Item 1 Variable	Maralixibat N=13	Placebo N=16	Maralixibat N=13	Placebo N=16	Maralixibat N=13	Placebo N=16
Baseline (pretreatment)	0	0	0	0	0	0
Week 18 (pre-randomized treatment)	0	0	0	0	0	0
RWD Period						
Week 19	1 (8%)	1 (6%)	2 (15%)	1 (6%)	1 (8%)	1 (6%)
Week 20	0	0	0	0	0	0
Week 21	0	0	0	0	0	0
Week 22	0	0	1 (8%)	0	0	0

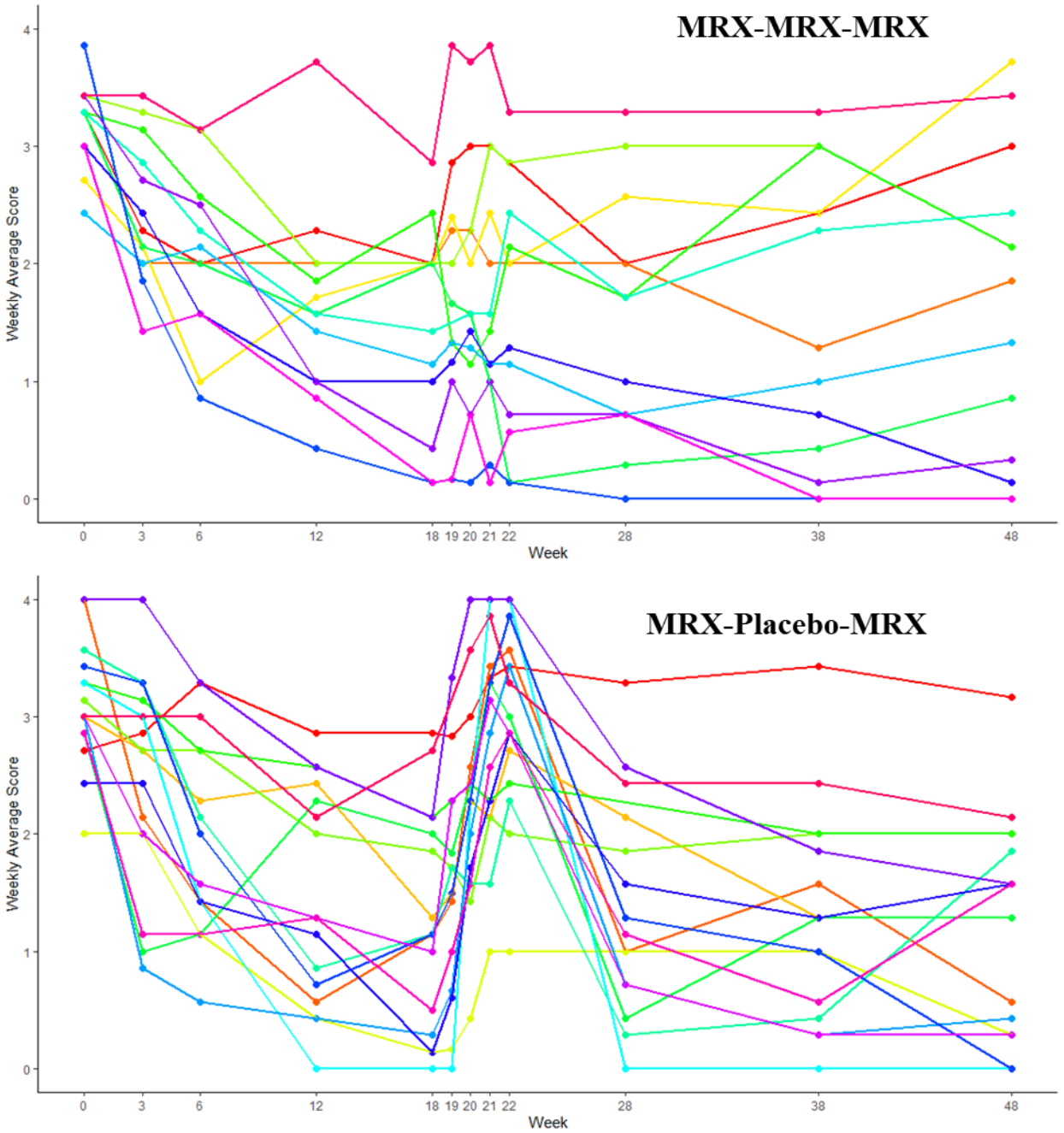
Source: Reviewer's analysis using the Applicant-submitted datasets adqs.xpt and adqs2.xpt.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); RWD, randomized withdrawal

Patient-Level Plots

Figure 45 presents patient trajectories using the weekly average of the worst daily ItchRO(Obs) scratching severity scores over the 48-week study period. Each colored line represents the outcome of a single patient. Patients randomized to maralixibat during the RWD period are depicted in the top plot; patients randomized to placebo during the RWD period are depicted in the bottom plot.

There is a clear trend of patients randomized to placebo having worsening pruritus during the RWD period, and then improving upon re-initiation of maralixibat treatment. While there appears to be a slight worsening of pruritus in the RWD period for patients randomized to maintain maralixibat treatment, this worsening is not as severe as those in the placebo group. This apparent worsening may be partially due to natural fluctuations in pruritus severity and a "reverse placebo effect" where the caregivers' pruritus assessment may be affected by knowledge that there is a chance that the patient was withdrawn from active treatment.

Figure 45. Patient Trajectory Plots from Baseline to Week 48, Weekly Average of Worst Daily ItchRO(Obs) Scratching Severity Scores (Observed Data), Study LUM001-304



Source: Reviewer's analysis using the Applicant-submitted dataset adqs2.xpt.
Abbreviations: ItchRO(Obs), Itch Reported Outcome (Observer); MRX, maralixibat

Analyses Using the Applicant's Strategy to Handle the Treatment Interruption of Patient LUM001-304-(b) (6)

Table 134, Table 135, and Table 136 present sensitivity analyses for the analyses in Table 11, Table 16, and Table 12, respectively, using the Applicant's strategy to handle the treatment interruption of Patient LUM001-304 (b) (6) instead of the treatment policy strategy (refer to

Section II.6.2.1.3). These sensitivity analyses had similar results and the same conclusions as the main analyses.

Table 134. Results for the Weekly Average of Worst Daily ItchRO(Obs) Scratching Severity Scores in the RWD Period, Applicant's Main Analysis, Study LUM001-304

ItchRO(Obs) Item 1 Variable	Maralixibat N=13	Placebo N=16	LS Mean Difference
Week 18 to Week 22			
Week 18*, Mean (SE)	1.5 (0.2)	1.3 (0.2)	NA
Week 22, LS Mean (95% CI)	1.6 (1.1, 2.1)	3.1 (2.7, 3.5)	
Change from Week 18 to Week 22, LS Mean (95% CI)	0.2 (-0.3, 0.7)	1.7 (1.3, 2.2)	-1.5 (-2.2, -0.9)
Baseline to Week 22			
Baseline, Mean (SE)	3.1 (0.1)	3.1 (0.1)	NA
Week 22, LS Mean (95% CI)	1.6 (1.1, 2.2)	3.1 (2.6, 3.5)	
Change from baseline to Week 22, LS Mean (95% CI)	-1.5 (-2.0, -1.0)	-0.04 (-0.5, 0.4)	-1.4 (-2.1, -0.7)

Source: Clinical study report LUM001-304 Tables and Figures document (page 704 [Table 14.2.10.2]); findings reproduced and supplemented by the statistical reviewer using the adqs.xpt dataset.
Results are based on ANCOVA models adjusted for Week 18 or baseline, respectively. There were no missing weekly observations at Week 18 or Week 22.
* Week 18 results are from the open-label treatment period and may be subject to bias, because patients and caregivers were aware that patients were receiving active treatment.
Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; ItchRO(Obs), Itch Reported Outcome (Observer); LOCF, last observation carried forward; LS, least squares; MRX, maralixibat; NA, not applicable; RWD, randomized withdrawal; SE, standard error

Table 135. Results for the Weekly Average of ItchRO(Obs) Scratching Severity Scores in the RWD Period Using Various Daily Summaries, Applicant's Main Analysis, Study LUM001-304

ItchRO(Obs) Item 1 Variable	Maralixibat N=13	Placebo N=16	LS Mean Difference
Change from baseline to Week 22, LS Mean (95% CI)			
Worst daily score	-1.5 (-2.0, -1.0)	-0.04 (-0.5, 0.4)	-1.4 (-2.1, -0.7)
Morning score*	-1.3 (-1.9, -0.8)	-0.08 (-0.6, 0.4)	-1.2 (-2.0, -0.5)
Evening score	-1.3 (-1.8, -0.8)	-0.02 (-0.5, 0.4)	-1.3 (-1.9, -0.6)
Average of AM and PM score	-1.3 (-1.8, -0.8)	-0.05 (-0.5, 0.4)	-1.3 (-1.9, -0.6)
Change from Week 18 to Week 22, LS Mean (95% CI)			
Worst daily score	0.2 (-0.3, 0.7)	1.7 (1.3, 2.2)	-1.5 (-2.2, -0.9)
Morning score*	0.3 (-0.2, 0.8)	1.7 (1.2, 2.1)	-1.4 (-2.0, -0.7)
Evening score	0.2 (-0.2, 0.7)	1.7 (1.3, 2.1)	-1.5 (-2.1, -0.9)
Average of AM and PM scores	0.2 (-0.2, 0.7)	1.7 (1.3, 2.1)	-1.5 (-2.1, -0.8)

Source: Clinical study report LUM001-304 Tables and Figures document (pages 704 [Table 14.2.10.2], 97 [Table 14.2.1.2], 502 [Table 14.2.2.2], and 650 [Table 14.2.9.2] for worst daily, morning, evening, and average daily results, respectively); findings reproduced by the statistical reviewer using the adqs.xpt dataset.
Results are based on an ANCOVA model adjusted for baseline or Week 18, respectively.
* LOCF was used to impute one missing morning score for Patient LUM001-304-(b) (6)
Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; ItchRO(Obs), Itch Reported Outcome (Observer); LOCF, last observation carried forward; LS, least squares; MRX, maralixibat; RWD, randomized withdrawal

Table 136. Results for the Weekly Average of Worst Daily ItchRO(Pt) Scratching Severity Scores in the RWD Period, Applicant's Main Analysis, Study LUM001-304

ItchRO(Obs) Item 1 Variable	Maralixibat N=5	Placebo N=9	LS Mean Difference
Week 18 to Week 22			
Week 18*, Mean (SE)	0.9 (0.3)	0.9 (0.3)	NA
Week 22, LS Mean (95% CI)	0.8 (0, 1.6)	2.9 (2.3, 3.5)	
Change from Week 18 to Week 22, LS Mean (95% CI)	-0.1 (-1.0, 0.7)	2.0 (1.4, 2.6)	-2.1 (-3.1, -1.1)
Baseline to Week 22			
Baseline, Mean (SE)	2.9 (0.2)	3.1 (0.2)	NA
Week 22, LS Mean (95% CI)	0.8 (0, 1.6)	2.9 (2.3, 3.5)	
Change from baseline to Week 22, LS Mean (95% CI)	-2.2 (-3.0, -1.4)	-0.1 (-0.7, 0.5)	-2.1 (-3.1, -1.1)

Source: Clinical study report LUM001-304 Tables and Figures document (page 902 [Table 14.2.16.2]); findings reproduced by the statistical reviewer using the adqs.xpt dataset.
Results are based on an ANCOVA model adjusted for Week 18 or baseline, respectively. There were no missing weekly observations at Week 18 or Week 22.
* Week 18 results are from the open-label treatment period and may be subject to bias, because patients and caregivers were aware that patients were receiving active treatment.
Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; ItchRO(Pt), Itch Reported Outcome (Patient); LS, least squares; MRX, maralixibat; NA, not applicable; RWD, randomized withdrawal; SE, standard error

17. Clinical Safety: Additional Information and Assessment

In humans, 600 mg per day of BA synthesis is needed to replenish losses of BA. Six to ten cycles per day results in 20 to 30 µmol/day. A small percentage of BA can be reabsorbed via passive or carrier-mediated transport in the small intestine. Also, 3 to 5% of BA that is secreted escapes intestinal absorption and is excreted in feces.

Table 137. Names and Kinetics of Individual Bile Acids in Human

Common Name	Type		Pool size (mg)	Daily synthesis (mg)
Cholic acid	Primary	Synthesized from cholesterol by	500–1500	180–360
Chenodeoxycholic acid	Primary	liver	500–1400	100–250
Deoxycholic acid	Secondary	Produced in intestine from	200–1000	
Lithocholic acid	Secondary	action of microorganisms on primary bile acids	50–100	
Total			1250–4000	280–610

Source: Bile acid malabsorption. British Medical Bulletin, page 82.

There are two pathways for bile acid synthesis: classical pathway (using microsomal cholesterol 7α hydroxylase or CYP7A1, the rate limiting step in BA synthesis) and alternate pathway (mitochondrial sterol 27-hydroxylase or CYP27A1). The classical pathway is the rate limiting step of BA synthesis. The classical pathway occurs in the liver and 2 primary BA are produced, cholic acid and chenodeoxycholic acid (Hofmann 1967).

The primary BA are conjugated with glycine (majority for humans) or taurine and secreted from the biliary tree into the duodenum for absorption/processing of food. Conjugation results in high luminal micellar concentration of BA thus facilitating lipid digestion and absorption in the small

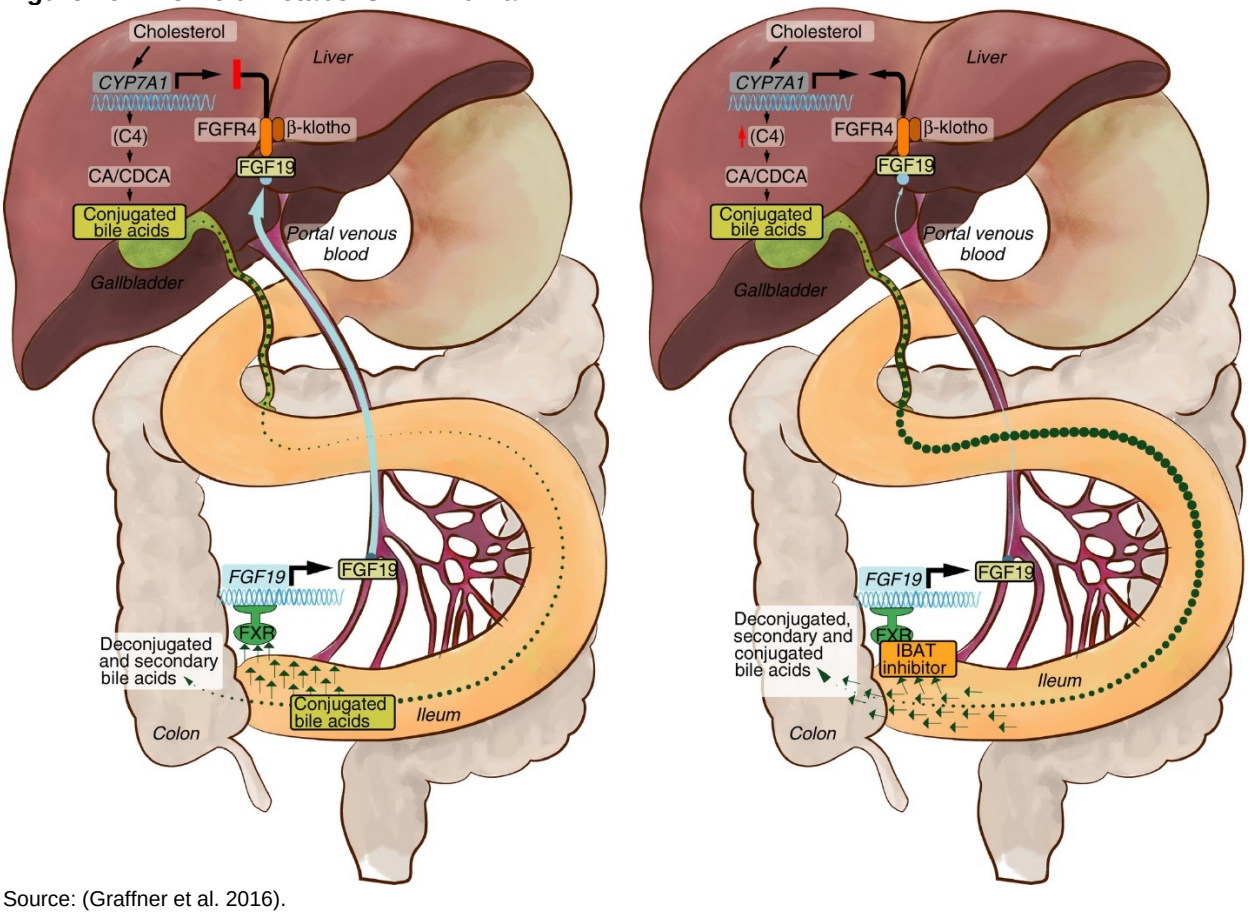
intestines. Cholestasis, therefore, is associated with impaired lipid homeostasis and fat-soluble vitamin deficiencies. See Figure 46.

A homeostatic mechanism maintains and regulates BA, such that BA reabsorption from the small intestines to the liver inhibits their own synthesis via negative feedback. BA binds to nuclear farnesoid X receptor in the liver, thereby inducing expression of small heterodimer partner, which inhibits expression of liver receptor homologue and CYP7A1 is suppressed. In addition, intestinal FGF-15 can function as a secretory signaling on the liver through the hepatic FGF receptor 4.

Other control mechanisms that assist in maintaining BA homeostasis include:

- BA in liver can activate the liver FGF-19/ FGF receptor 4 to inhibit BA synthesis and prevent the toxic accumulation of toxic bile in the liver.
- BA production is associated with diurnal rhythm and appears to follow postprandial release of BA by approximately 90 to 180 min after peak serum BA.
- FGF-19-mediated suppression of BA synthesis is supported by the temporal relationship between FGF-19 and C4.

Figure 46. Bile Acid Metabolism in Human



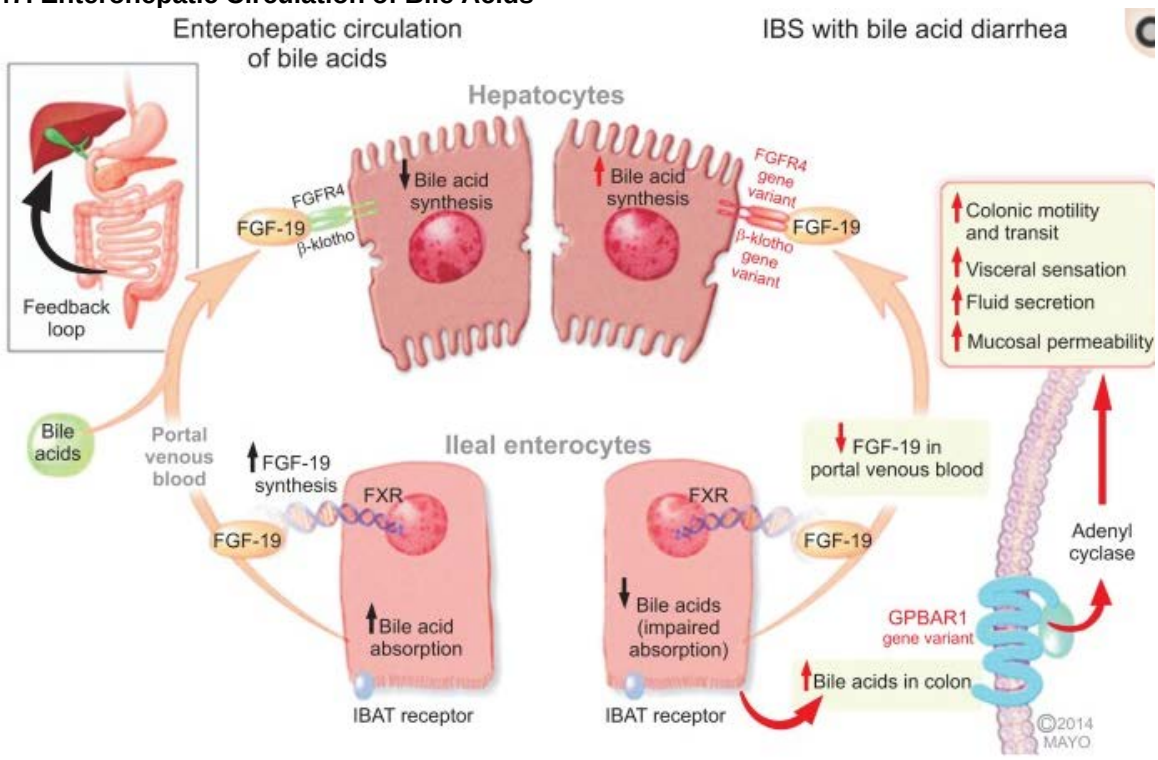
Source: (Graffner et al. 2016).

Enterohepatic circulation of bile acids: bile acids are absorbed by ileal enterocytes via IBAT receptor mediated process. Intracellular bile acids activate farnesoid-gamma receptors to increase

FGF-19 synthesis. In the portal circulation, FGF-19 downregulates hepatocyte bile acid synthesis. See Figure 47.

Genetic variations of FGFR4 or beta-klotho and disorders of FGF-19 synthesis by ileal enterocytes result in increased bile acid concentrations in the colon, activation of 5-hydroxytrytamine, colonic motility stimulation, increased fluid secretion and activation of visceral sensations. These physiologic processes contribute to the GI AEs of IBAT inhibitors (Camilleri 2015).

Figure 47. Enterohepatic Circulation of Bile Acids



Source: (Camilleri 2015)
Abbreviations: IBAT, intestinal bile acid transporter; IBS, irritable bowel syndrome

Fat-Soluble Vitamins, Serum Bile Acids, and Fractures

FSV deficiencies are common in cases of chronic cholestasis, whereby there is poor micellar solubilization of fat, hence decreased intestinal FSV absorption.

Upon review of the data provided by the Applicant in an Information Request, in general FSV levels did not correlate with the degree of sBA reduction, except for 25-OH vitamin D levels. Reduction of sBA by ≥50% was associated with a lower frequency of insufficient 25-OH vitamin D levels (2/12, 16.7%) at Week 48 compared with (6/15, 40%) cases of insufficient 25-OH vitamin D levels at Week 48 after a <50% reduction in sBA. See Table 138.

Table 138. Distribution of Fractures and FSV Deficiencies by sBA Response at Week 48, Study LUM001-304

	Patients with >=50% reduction of sBA at Week 48 (N = 12)	Patients with <50% reduction of sBA at Week 48 (N = 15)
Total Fractures	**1 (8.3%)**	**0**
Upper limb Fractures	1 (8.3%)	0
Lower limb Fractures	0	0
*Vitamin A Deficiency	0	1 (6.7%)
*Vitamin D Deficiency	1 (8.3%)	0
*Vitamin K Deficiency	0	0
*Vitamin E Deficiency	0	0

*Reported as an AE by the site investigator
"Vitamin X deficiency" includes 3 preferred terms: (i) Vitamin X deficiency, (ii) Vitamin X abnormal, and (iii) Vitamin X decreased.
Preferred term of "Forearm fracture" from subject (b) (6) (see Appendix 1) accounts for the lone fracture event.
Source: page 4, Clinical Information Amendment Submitted on May 7, 2021
Abbreviations: AE, adverse event; FSV, fat-soluble vitamin; sBA, serum bile acids

After maralixibat was commenced, a consistent decline in sBA occurred from a mean (range) of 335.3 µmol/L (77.07 µmol/L to 1014 µmol/L) to 104.59 µmol/L (8.59 µmol/L to 324.47 µmol/L) after a mean (range) treatment period of 1068 days (107 days to 2052 days) of exposure to maralixibat. See Table 139.

Table 139. Reported Fractures Across the ALGS Pooled Population (N=86)

Subject ID	Reported event (PT)	Start Day (Study Day)	Serious (Y/N)	Severity (Grade)	Action Taken	Investigator Causality	Outcome	sBA Baseline µmol/L	sBA Low µmol/L (Day after start of MRX)	sBA High µmol/L (Day after start of MRX)
(b) (6)	Tibia fracture	951	N	2	Dose not changed	Possibly related	Recovered/Resolved	242.74	101.07 (Day 341)	264.39 (Day 1022)
	Rib fracture	118	N	1	Dose not changed	Not-related	Recovered/Resolved	1014.22	342.47 (Day 348)	1160.54 (Day 15)
	Hand fracture	781	N	1	Dose not changed	Not-related	Recovered/Resolved	151.25	8.59 (Day 763)	99.03 (Day 61)
	Humerus fracture	2068	Y	2	Dose not changed	Not-related	Recovered/Resolved with sequelae	293.76	87.82 (Day 1672)	372.33 (Day 168)
	Pathological fracture	2075	Y	2	Dose not changed	Not-related	Recovered/Resolved with sequelae	293.76	87.82 (Day 1672)	372.33 (Day 168)
	Forearm fracture	463	Y	2	Dose not changed	Not-related	Recovered/Resolved	77.07	41.39 (Day 525)	87.3 (Day 175)
	Hand fracture	540	N	2	Dose not changed	Not-related	Recovered/Resolved	573.91	298.17 (Day 107)	648.79 (Day 178)
	Clavicle fracture	373	N	1	Dose not changed	Not-related	Recovered/Resolved	228	53.94 (Day 2052)	304.03 (Day 14)
	Forearm fracture	1479	Y	3	Dose not changed	Not-related	Recovered/Resolved	239.42	21.3 (Day 1602)	135.17 (Day 158)*
	Forearm fracture	1571	Y	3	Drug interrupted	Not-related	Recovered/Resolved	239.42	21.3 (Day 1602)	135.17 (Day 158)*

* Note: Patient LUM001-304-(b) (6) was randomized to placebo during the randomized withdrawal period and the maximum sBA reported was at Week 22.

Source: page 5, Clinical Information Amendment Submitted on May 7, 2021
Abbreviations: ALGS, Alagille syndrome; MRX, maralixibat; PT, preferred term; sBA, serum bile acids

There was variability in the observed changes in FSV levels in response to a <50% compared with a ≥50% decline in sBA, as shown in Table 140.

Table 140. Fat-Soluble Vitamin Level Shifts by sBA Response at Week 48, Study LUM001-304

Lab Parameter	Range Category	Patients with >=50% reduction of sBA at Week 48 (N = 12)		Patients with <50% reduction of sBA at Week 48 (N = 15)	
		Baseline	Week 48	Baseline	Week 48
Vitamin A (ug/dL)	Insufficient (< 20 ug/dL)	0	1 (8.3%)	0	1 (6.7%)
	Sufficient (20 to 77 ug/dL)	11 (91.7%)	11 (91.7%)	14 (93.3%)	12 (80.0%)
	Excess (> 77 ug/dL)	0	0	0	2 (13.3%)
	Missing	1 (8.3%)	0	1 (6.7%)	0
25-Hydroxyvitamin D (ng/mL)	Insufficient (< 20 ng/mL)	7 (58.3%)	2 (16.7%)	3 (20.0%)	6 (40.0%)
	Sufficient (>= 20 to 96 ng/mL)	5 (41.7%)	10 (83.3%)	11 (73.3%)	9 (60.0%)
	Excess (> 96 ng/mL)	0	0	1 (6.7%)	0
	Missing	0	0	0	0
Alpha Tocopherol (mg/dL)	Low	1 (8.3%)	0	0	1 (6.7%)
	Normal	1 (8.3%)	2 (16.7%)	8 (53.3%)	7 (46.7%)
	High	9 (75.0%)	10 (83.3%)	6 (40.0%)	7 (46.7%)
	Missing	1 (6.7%)	0	1 (6.7%)	0
Prothrombin Intl. Normalized Ratio	Sufficient (< 1.2)	11 (91.7%)	12 (100.0%)	11 (73.3%)	10 (66.7%)
	Indeterminate (>= 1.2 to 1.5)	0	0	4 (26.7%)	4 (26.7%)
	Possibly Insufficient (> 1.5)	1 (8.3%)	0	0	0
	Missing	0	0	0	1 (6.7%)
Ratio of Alpha Tocopherol to the sum of Cholesterol and Triglycerides (mg/g)	Insufficient (<= 0.8 mg/g)	0	0	1 (6.7%)	1 (6.7%)
	Sufficient (> 0.8 to < 3.5 mg/g)	9 (75.0%)	9 (75.0%)	11 (73.3%)	13 (86.7%)
	Excess (>= 3.5 mg/g)	2 (16.7%)	3 (25.0%)	2 (13.3%)	1 (6.7%)
	Missing	1 (8.3%)	0	1 (6.7%)	0

Baseline Vitamin A and Vitamin E results are missing for responder subject (b) (6) and non-responder subject (b) (6) Week 48 INR result is missing for non-responder subject (b) (6)

Source: page 6, Clinical Information Amendment Submitted on May 7, 2021
Abbreviations: INR, international normalized ratio; sBA, serum bile acids

Vitamin D deficiency was associated with fractures and occurred in one patient whose sBA level declined by >50%. Strong conclusions cannot be made because of the small numbers of subjects; however, dose adjustment of maralixibat and vitamin D supplementation could be considered in cases where the sBA level has fallen by >50% and vitamin D deficiency is diagnosed.

Liver Transplantation

Five subjects in the Integrated Summary of Safety population underwent liver transplantation. Liver transplantation occurred during treatment with maralixibat for a mean (range) of 1230 days (437 to 1718 days). No liver transplants were a result of acute liver failure or acute decompensation or drug-induced liver injury related to maralixibat. The indications for liver transplantation were disease progression (two), symptomatic disease with cholestasis, xanthomas, pruritus and growth retardation (two), and hepatocellular carcinoma (one).

Laboratory data at the time point closest to liver transplantation revealed:

- Thrombocytopenia in two subjects with platelet counts of 139,000 and 149,000/µL, respectively.
- Hyperbilirubinemia with a mean (range) bilirubin of 8.68 (1.2 to 15.4) mg/dL.
- At the time of liver transplantation, subject LUM001-303-(b) (6) discontinued maralixibat after voluntarily withdrawing from the study.
- LUM001-301-(b) (6) discontinued maralixibat because of hyperbilirubinemia (total bilirubin 12.0 mg/dL; direct bilirubin, 8.0 mg/dL)
- According to the Applicant, eight additional subjects underwent liver transplantation after discontinuing the study.

18. Mechanism of Action/Drug Resistance: Additional Information and Assessment

Not applicable.

19. Other Drug Development Considerations: Additional Information and Assessment

19.1. Division of Pediatric and Maternal Health Review

Consult findings from the Division of Pediatric and Maternal Health (DPMH) are summarized below. The consult was entered in the Document Archiving, Reporting, and Regulatory Tracking System on June 28, 2021. Labeling recommendations for Sections 8.1 and 8.2 were incorporated into the final Label (refer to Section 21).

Animal Data

Pregnancy

Maralixibat has low absorption following oral administration, and maternal use at the proposed clinical dose is not expected to result in measurable fetal exposure to maralixibat. There are no data on the use of maralixibat in humans during pregnancy. In animal reproduction studies, maralixibat administered in pregnancy did not result in embryotoxicity or structural malformation.

Based on its mechanism of action, maralixibat may inhibit the absorption of dietary fats and lipid-soluble vitamins as well as bile salts. Alagille syndrome is often associated with malnutrition, and FSV supplementation is a part of the standard of care. FSVs are necessary for normal development in a growing fetus, and maralixibat use may add further insult to an already depleted FSV store in a pregnant patient with Alagille syndrome. FSV depletion in pregnancy may be detrimental to a growing fetus; therefore, based on discussion with the Division of Hepatology (DHN) and Nutrition Clinical Team, DPMH agrees with adding language about

monitoring for FSV deficiency in pregnant patients and supplementing FSV if necessary under 8.1, Clinical Considerations.

Alagille syndrome is a rare disease with an incidence of 1 in 30,000 to 1 in 70,000. Only 11 successful pregnancies have been reported to date. Additionally, systemic absorption following oral administration of maralixibat is low, and maternal use is not expected to result in fetal exposure to maralixibat. Therefore, DPMH does not recommend a postmarketing pregnancy study at this time.

Lactation

Maralixibat has low absorption following oral administration, and breastfeeding is not expected to result in measurable exposure of the infant to maralixibat at the clinical dose. There are no data on the presence of maralixibat in human milk, the effects of the drug on the breastfed infant, or on milk production.

Although there were no animal lactation studies, pre- and post-natal developmental studies were conducted with maralixibat. There were no AEs on postnatal development; however, maralixibat was detected in rat pup plasma. In discussion with the DHN Pharmacology Toxicology Team, the team noted that the presence of maralixibat in rat pup plasma was likely due to transfer from rat milk and not from prenatal exposure. The lactating rats were given doses of maralixibat at human exposure multiples that were 257- to 956-fold the maximum recommended human dose of 28.5 mg per day, which would explain the higher concentrations of maralixibat in rat pup plasma. The clinical significance of this finding is uncertain.

Because there is low absorption of the drug after oral administration, breastfeeding is not expected to result in significant exposure of the infant to maralixibat. Therefore, DPMH does not recommend a lactation study at this time.

Females and Males of Reproductive Potential

There are no reports of maralixibat adversely effecting human fertility. Based on animal studies, maralixibat has no effect on male fertility and no effects on mating in either sex. There are no data to suggest maralixibat interacts with systemic hormonal contraceptive. DPMH recommends omitting subsection 8.3.

20. Data Integrity-Related Consults (Office of Scientific Investigations, Other Inspections)

Overall Assessment of Findings and Recommendations

Clinical data from Study LUM001-304 were submitted to the FDA in support of NDA 214662 for the use of maralixibat for the treatment of cholestatic pruritis in patients with ALGS 1 year of age and older. Three clinical investigators who participated in Study LUM001-304—Dr. Loreto Hierro [Site 040], Dr. Dorota Gliwicz-Miedzinska [Site 080], Dr. Etienne Sokal [Site 090]—and the Applicant Mirum Pharmaceutical Inc. were inspected.

Dr. Alastair Baker [Site 001], United Kingdom was initially selected for clinical site inspection. However, logistics related to coronavirus disease-2019 pandemic concerns resulted in cancellation of the site inspection.

The inspections found no significant regulatory violations at either the three investigator sites or the Applicant, Mirum Pharmaceutical Inc. The Applicant's submitted clinical data listings were verifiable against source records, with no discrepancies identified. The clinical data generated by the inspected investigators appear to be reliable. Based on the results of these inspections, Study LUM001-304 appears to have been conducted adequately, and the clinical data generated from the inspected entities appear to be reliable in support of this NDA.

21. Labeling Summary of Considerations and Key Additional Information

For the final labeling, please see the finalized PI submitted to 1.14 of the docuBridge. The changes in the PI are summarized below.

The Division of Records Management and DHN agree that the potential risks of maralixibat can be adequately managed in the postmarketing setting through labeling alone, and that a risk evaluation and mitigation strategy is not necessary to ensure that the drug's benefit will outweigh its risks.

Table 141 summarizes the major changes (additions, deletions, or modifications) made by the FDA to the PI proposed by the Applicant. The rationale for the FDA recommendation is provided in the table based on the evidence from Study LUM001-304 and supportive studies, regulatory principles, literature review, and interactive review with the Applicant. Refer to Sections II.6 and II.7 for more information. Two additional labeling documents, *Instructions for Use* and *Patient Information* were drafted during review of the NDA to assist patients and caregivers in administering the liquid solution.

Table 141. FDA Recommendations to the Prescribing Information

Section and Title	FDA Recommendation	Rationale
1. Indication and Usage	No change	
2. Dosage and Administration	• Updated dosing to (b) (4) 380 mcg/kg. • Addition of separate dosing by 4 h for patients taking bile acid binding resins. • Updated storage requirements after opening to allow for room temperature storage up to 45 days. • Added Section 2.4 for dose modification for management of adverse events.	• Based on salt naming policy to express dose in the base, (b) (4) • DDI studies not performed with bile acid binding resins, and bile acid binding resins not used with maralixibat in drug development program. • Updated stability testing demonstrated stability after opening for up to 45 days. • Provided guidance for management of dosing and dose interruption in the setting of liver test abnormalities or hepatic decompensation.

Section and Title	FDA Recommendation	Rationale
3. Dosage Forms and Strengths	• Changed (b) (4) to 9.5 mg of maralixibat.	• Based on salt naming policy to express dose in the base, (b) (4)
4. Contraindications	• No change.	• No contraindications were identified during the review.
5. Warnings and Precautions	• Added three key risks observed during the study: • Liver test abnormalities. • Fat-soluble vitamin deficiency. • GI: Diarrhea, abdominal pain and vomiting.	• These adverse reactions were commonly observed in the studies. These could lead to significant medical consequences. The three risks should be monitored and treatment to be interrupted or discontinued based on severity of clinical condition.
6. Adverse Reactions	• Addition of Table 2, adverse reactions occurring in >5% of patients treated with maralixibat.	• Table is a summary of the drug development program, without placebo comparator given the majority of exposure was in an open-label manner.
7. Drug Interactions	• Added potential interaction with bile acid-binding resins. • Updated recommendation to monitor drug effects of OATP2B1 substrates (e.g., statins).	• To avoid drug-drug interactions in the gut lumen. • A decrease in oral absorption of OATP2B1 substrates due to OATP2B1 inhibition in the GI tract cannot be ruled out.
8. Use in Specific Populations	• Added consideration of fat-soluble vitamin deficiency in the setting of pregnancy and lactation. • Removed (b) (4)	• Although maralixibat is not expected to be absorbed in a manner that would lead to detectable levels in breast milk or direct effects on fetal development, a reminder was added to monitor for FSV levels during pregnancy and lactation. • (b) (4)
9. Drug Abuse and Dependence	• Omitted by the Applicant.	• No data.
10. Overdosage	• Updated to include propylene glycol content per milliliter.	• Important in the setting of potential overdose due to safety concern with propylene glycol in young children.
11. Description	• Updated class (b) (4) to ileal bile acid transporter (IBAT).	• Maintain consistency in nomenclature with first-in-class approved IBAT inhibitor.

Section and Title	FDA Recommendation	Rationale
12. Clinical Pharmacology	• Removed (b) (4) • Removed (b) (4). • Updated information on food effect • Added section on elimination. • Modified the drug interaction studies section.	• (b) (4) not pertinent for labeling. • Updated language regarding potential interaction with OATP2B1 substrates and inhibition of absorption in the GI tract (to be consistent with Section 7). • Updated sections to be consistent with clinical pharmacology labeling guidance.
13. Nonclinical Toxicology	• Simplified content for clarity.	• To improve readability.
14. Clinical Studies	• Revised the text for description of Trial 1. • Changed the efficacy table and removed (b) (4) • Deleted sBA, xanthoma, clinician scratch score results from this section and moved it to Section 12.	• To provide key trial information and increase readability. • Removed (b) (4) • (b) (4) • sBA level is considered a pharmacodynamic marker; therefore, moved to Section 12.
15. References	• Omitted by the Applicant.	• No need for references.
16. How Supplied/ Storage and Handling	• Updated storage requirements after bottle opening.	• Changes based on updated stability testing data provided by the Applicant during the NDA review process.
17. Patient Counseling Information	• Added information for risks. • Added information regarding potential interaction with bile acid-binding resins. • Added information regarding updated storage requirements.	• To be consistent with modified PI.

Abbreviations: DDI, drug-drug interaction; GI, gastrointestinal; FDA, Food and Drug Administration; NDA, new drug application; OATP, organic anion transporting peptide; PI, prescribing information; sBA, serum bile acids;

22. Postmarketing Requirements and Commitments

The following postmarketing requirement will be issued at the time of approval:

4501-1: Conduct a 2-year carcinogenicity study in rats.

Study Completion:	10/2022
Final Report Submission:	10/2023

The following Section-506B postmarketing commitments will be issued at the time of approval:

4501-2: Provide a final study report capturing the comprehensive safety experience of ALGS patients treated in trial MRX-800.

The timetable you submitted on September 13, 2021, states that you will conduct this study according to the following schedule:

Interim Report Submission:	07/2022
Study/Trial Completion:	09/2023
Final Report Submission:	01/2024

4501-3: Conduct a 5-year registry-based study to collect data on the health of patients chronically treated with Livmarli (maralixibat). Report yearly on the following safety endpoints:

- Incidence of biliary diversion surgery, liver transplantation, and all-cause mortality
- Assessment of growth and development
- Incidence of fat-soluble vitamin deficiencies and their long-term sequelae (e.g., bone fracture, bleeding episodes).

Draft Protocol Submission:	01/2022
Final Protocol Submission:	07/2022
Interim Report:	12/2023
Interim Report:	12/2024
Interim Report:	12/2025
Interim Report:	12/2026
Study Completion:	09/2027
Final Report Submission:	03/2028

The following postmarketing commitment, not subject to reporting requirements under Section 506B, will be issued at the time of approval:

4501-4: Provide data from an extractables study (b) (4) used during commercial manufacturing or a leachables study on the bulk solution. The extractables or leachables study conditions should adequately represent the worst-case manufacturing conditions (temperature, time of exposure, etc.) and should consider non-volatile, semivolatile, and volatile type leachables. Demonstrate the analytical methods are suitable for detecting leachables from the (b) (4) manufacturing equipment and are sufficiently sensitive to detect and quantitate the leachables at or above AET. Include a discussion of the overall control strategy ((b) (4) during manufacturing) to be implemented during commercial

manufacturing to limit the amount of leachables from the manufacturing equipment in the final drug product.

Final Report Submission as CBE-0 supplement: 10/31/2021

23. Financial Disclosure

Table 142. Covered Clinical Studies: Studies LUM001-301, -302, -303, -304, and -305

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 141		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 5		
Four principal investigators (PIs) and one subinvestigator with disclosable financial interests/arrangements who conducted Studies LUM-001-301, -302, -303, -304, and -305: • (b) (6) received an unrestricted educational grant for ~$86,000. Dr. (b) (6) site enrolled 11 of 20 patients in Study LUM001-302 (including 11 of 19 who rolled over into the LTE, LUM001-303) and 3 of 31 subjects in Study LUM001-304. Serum bile acids are objectively reported and the ItchRO(Obs) is directly reported by the participant's caregiver in an electronic diary. Zero serious adverse events (SAEs) have occurred at Dr. (b) (6) site. • (b) (6) for Studies LUM001-302 and LUM001-303 (Dr. (b) (6) was the PI, see above). Dr. (b) (6) was paid a total of $97,598 by the Applicant for consulting work with Mirum Pharmaceuticals. • (b) (6) Dr. (b) (6) was paid a total of $53,894 by the Applicant for consulting work with Mirum Pharmaceuticals. Dr. (b) (6) institution received an unrestricted educational grant for $434,000. Dr (b) (6) site enrolled 4 of 34 subjects in Study LUM001-301 (including 4 of 34 who rolled over into the LTE, LUM001-305). Serum bile acids are objectively reported and the ItchRO(Obs) is directly reported by the participant's caregiver in an electronic diary. Zero SAEs have occurred at Dr. (b) (6) site. • (b) (6) Dr. (b) (6) was paid a total of $5,363 by the Applicant for consulting work with Mirum Pharmaceuticals. Dr. (b) (6) institution received an unrestricted educational grant for $75,000. Dr (b) (6) site enrolled 2 of 37 subjects in Study LUM001-301, of which 2 of 34 rolled over into the LTE study, LUM001-305. Serum bile acids are objectively reported and the ItchRO(Obs) is directly reported by the participant's caregiver in an electronic diary. One SAE occurred at Dr. (b) (6) site and was reviewed by the medical monitor. • (b) (6) Dr. (b) (6) was paid a total of $1000 for consulting work with Mirum Pharmaceuticals. Dr. (b) (6) institution received ~$60,182 for funding for preclinical research. Dr (b) (6) site enrolled 5 of 37 subjects in Study LUM001-301, of which 4 of 34 rolled over into the LTE study, LUM001-305. Serum bile acids are objectively reported and the ItchRO(Obs) is directly reported by the participant's caregiver in an electronic diary. Two SAEs occurred at Dr. (b) (6) site and were reviewed by the medical monitor.		

If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 5 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator: 0 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

24. References

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25. Review Team

Table 143. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory Project Manager	Navdeep Bhandari and Lisa Skarupa
Nonclinical Reviewer	Fang Cai
Nonclinical Team Leader	David Joseph
Nonclinical Reviewer (carcinogenicity)	Frederic Moulin
Nonclinical (statistical)	**Reviewer:** Zhuang Miao **Team Leader:** Karl Lin
Clinical Pharmacology Reviewer(s)	Anand Balakrishnan Dilara Jappar
Clinical Pharmacology Team Leader	Insook Kim
Clinical Pharmacology	**PBPK Reviewer:** Ying-Hong Wang **PBPK Team Leader:** Yuching Yang **QT Reviewer:** Giresh Bene **QT Team Leader:** Nan Zheng
Clinical Reviewer	Charmaine Stewart
Clinical Team Leader and Cross-Disciplinary Team Leader	George Makar
Statistical Reviewer	Rebecca Hager
Statistical Team Leader	George Kordzakhia
PFSS (Patient-Focused Statistical Support)	Monica Morell, Lili Garrard
COA (Clinical Outcome Assessment)	Yujin Chung, TL Onyeka Illoh, David Reasner
Division Director (pharm/tox)	Carmen Booker
Division Director (OCP)	Suresh Doddapaneni
Division Director (OB)	Laura Lee Johnson
Division Director (clinical)	Joseph Toerner
Office Director (signatory authority)	Julie Beitz

OB, Office of Biostatistics; OCP, Office of Clinical Pharmacology; PBPK Physiologically Based Pharmacokinetic

Table 144. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	RBPM: Oumou Barry, ATL: Hitesh Shroff Drug Product: Jane Chang, TL Hong Cai Drug Substance: Sam Bain, TL Donna Christner Microbiology: Karthik Krishnan, TL Yeissa Chabrier Rosello OPMA: Yan Zheng, TL David Anderson
OPDP	Meeta Patel, TL Katie Klemm
OSI	Zana Handy Marks, TL Min Lu
OSE RPM	Shawnetta Jackson
OSE/DEPI	Benjamin Booth, Joel Weissfeld TL Sukhminder Sandhu
OSE/DMEPA	Sarah Vee, TL Idalia Rychlik
OSE/DMEPA Human Factors	Matthew Barlow, TL Ebony Whaley
OSE/DRM	Donella Fitzgerald, TL Jacqueline Sheppard, AD Doris Auth
OSE/DPV	Jamie Klucken, TL Lisa Wolf
DPMH	RPM: Niquiche Guity; Regulatory TL: Rosemary Addy Maternal: Wenjie Sun (MO), Miriam Dinatale (TL) Pediatric: Ethan Hausman (MO), Shetarra Walker (TL)
OND Program OPS: Rare Pediatric Disease Priority Review Voucher	Pinakini Patel and Cathryn Lee, Sara Stradley, Maarika Kimbrell
Clinical Data Analyst	Salman Hosain, TL Jinzhong Liu

Abbreviations: DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DPMH, Division of Maternal and Pediatric Health; MO, Medical Officer; OND, Office of New Drugs; OPMA, Office of Pharmaceutical Manufacturing Assessment; OPQ, Office of Pharmaceutical Quality; OPS, Office of Pharmaceutical Science; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations; RBPM, Regulatory Business Process Manager; TL, Team Leader

Table 145 Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Pharmacology/Toxicology	David Joseph, PhD	OND/OII/DPT-II	5.1, 7.1, 8.4, 13.1.1, 13.1.2, 13.1.3, 13.2.1, 13.2.2, 13.2.3 ☐ Authored ☒ Contributed ☒ Approved
Team Leader	**Signature:** David B. Joseph -S Digitally signed by David B. Joseph -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300134835, cn=David B. Joseph -S Date: 2021.09.24 09:56:32 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Pharmacology/Toxicology	Fang Cai, PhD	OND/OII/DPT-II	5.1, 7.1, 8.4, 13.1.1, 13.1.2, 13.1.3, 13.2.1, 13.2.2, 13.2.3 (except 13.2.3.4) ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Fang Cai -S Digitally signed by Fang Cai -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Fang Cai -S, 0.9.2342.19200300.100.1.1=2000585678 Date: 2021.09.24 10:04:41 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Pharmacology/Toxicology	Frederic Moulin, PhD	OND/OII/DPT-II	13.2.3.4 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Frederic Moulin -S Digitally signed by Frederic Moulin -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001708658, cn=Frederic Moulin -S Date: 2021.09.23 15:28:08 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Pharmacology/Toxicology Deputy Director	Carmen D. Booker, PhD	OND/OII/DPT-II	5.1, 7.1, 8.4, 13.1.1, 13.1.2, 13.1.3, 13.2.1, 13.2.2, 13.2.3 ☐ Authored ☐ Contributed ☒ Approved
Tertiary Reviewer	**Signature:** Carmen D. Booker -S Digitally signed by Carmen D. Booker -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300171107, cn=Carmen D. Booker -S Date: 2021.09.24 11:18:05 -04'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Continued: Table 145 Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Statistical (Pharmacology/Toxicology)	Zhuang Miao, PhD	OB/DBVI	13.2.3.4 ☐ Authored ☒ Contributed ☒ Approved
Reviewer	**Signature:** Zhuang Miao -S Digitally signed by Zhuang Miao -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Zhuang Miao -S, 0.9.2342.19200300.100.1.1=2001275134 Date: 2021.09.24 08:35:44 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Statistical (Pharmacology/Toxicology)	Karl Lin, PhD	OB/DBVI	13.2.3.4 ☐ Authored ☒ Contributed ☒ Approved
Team Leader	**Signature:**		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Statistical (Hepatology and Nutrition)	George Kordzakhia, PhD	OTS/OB/DBIII	6.2.1.1, 6.2.1.3, 6.2.1.4, 6.3.4, 16.2, 16.3 ☐ Authored ☐ Contributed ☒ Approved
Team Leader	**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: 2021.09.24 10:13:58 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Statistical (Hepatology and Nutrition)	Rebecca Hager, PhD	OTS/OB/DBIII	6.2.1.1, 6.2.1.3, 6.2.1.4, 6.3.4, 16.2, 16.3 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Rebecca S. Hager -S Digitally signed by Rebecca S. Hager -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002201387, cn=Rebecca S. Hager -S Date: 2021.09.23 13:55:36 -04'00'		

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Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Statistical (Pharmacology/Toxicology)	Karl Lin, PhD	OB/DBVI	13.2.3.4 ☐ Authored ☒ Contributed ☒ Approved
Team Leader	**Signature:** Karl K. Lin -S Digitally signed by Karl K. Lin -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Karl K. Lin -S, 0.9.2342.19200300.100.1.1=1300041229 Date: 2021.09.24 08:14:04 -04'00'		

Continued: Table 145 Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Statistical	Laura Lee Johnson, PhD	OTS/OB/DBIII	6.2.1.1, 6.2.1.3, 6.2.1.4, 6.3.2, 6.3.3, 6.3.4, 6.3.5 16.1.2, 16.1.3, 16.1.4, 16.1.5, 16.2, 16.3 ☐ Authored ☐ Contributed ☒ Approved
Division Director	**Signature:** Laura L. Johnson -S Digitally signed by Laura L. Johnson -S Date: 2021.09.24 09:04:39 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Statistical (Patient Focused Statistical Support)	Lili Garrard, PhD	OTS/OB/DBIII	6.2.1.4, 6.3.3, 6.3.5, 16.1.2, 16.1.3, 16.1.4, 16.1.5 ☐ Authored ☒ Contributed ☒ Approved
Team Leader	**Signature:** Lili Garrard -S Digitally signed by Lili Garrard -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Lili Garrard -S, 0.9.2342.19200300.100.1.1=2001916551 Date: 2021.09.23 14:18:06 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Statistical (Patient Focused Statistical Support)	Monica Morell, PhD	OTS/OB/DBIII	6.2.1.4, 6.3.3, 6.3.5, 16.1.2, 16.1.3, 16.1.4, 16.1.5 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Monica C. Morell -S Digitally signed by Monica C. Morell -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2003141685, cn=Monica C. Morell -S Date: 2021.09.23 13:37:21 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Insook Kim, PhD	OTS/OCP/DIIP	5, 6.1, 8.1, 8.2,14.1, 14.2, 14.3, 14.4, 14.5 ☐ Authored ☒ Contributed ☒ Approved
Team Leader	**Signature:** Insook Kim -S Digitally signed by Insook Kim -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Insook Kim -S, 0.9.2342.19200300.100.1.1=1300416436 Date: 2021.09.24 08:48:52 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Anand Balakrishnan, PhD	OTS/OCP/DIIP	5, 6.1, 8.1, 8.2,14.1, 14.2, 14.3, 14.4, 14.5 ☒ Authored ☒ Contributed ☐ Approved
Reviewer	**Signature:** Anand Balakrishnan -S Digitally signed by Anand Balakrishnan -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001742021, cn=Anand Balakrishnan -S Date: 2021.09.24 10 43:17 -04'00'		

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Continued: Table 145 Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Ying-Hong Wang, PhD	OTS/OCP/DIIP	14.4 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Ying-hong Wang -S Digitally signed by Ying-hong Wang -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Ying-hong Wang -S, 0.9.2342.19200300.100.1.1=2002939330 Date: 2021.09.23 14:01:16 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Clinical Pharmacology	Yuching Yang, PhD	OTS/OCP/DIIP	14.4 ☐ Authored ☒ Contributed ☒ Approved
Team Leader	**Signature:** Yuching Yang -S Digitally signed by Yuching Yang -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yuching Yang -S, 0.9.2342.19200300.100.1.1=2000846164 Date: 2021.09.24 12:49:39 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Clinical Pharmacology	Suresh Doddapaneni, PhD	OTS/OCP/DIIP	Enter sections. ☐ Authored ☐ Contributed ☒ Approved
Division Director (Acting)	**Signature:** Suresh N. Doddapaneni -S Digitally signed by Suresh N. Doddapaneni -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300101327, cn=Suresh N. Doddapaneni -S Date: 2021.09.24 07:36:34 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Clinical Outcome Assessment	David Reasner, PhD	OND/ODES/DCOA	6.3.3, 6.3.5, 16.1.1, 16.1.5 ☐ Authored ☐ Contributed ☒ Approved
Division Director	**Signature:** David Reasner -S Digitally signed by David Reasner -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=David Reasner -S, 0.9.2342.19200300.100.1.1=2003187028 Date: 2021.09.24 13:02:39 -04'00'		
Discipline and Title or Role	**Reviewer Name**	**Office/Division**	**Sections Authored/ Acknowledged/ Approved[1]**
Clinical Outcome Assessment	Yujin Chung, PhD **Dr. David Reasner is signing on behalf of Dr. Yujin Chung**	OND/ODES/DCOA	6.3.3, 6.3.5, 16.1.1, 16.1.5 ☒ Authored ☒ Contributed ☐ Approved
Reviewer	**Signature:** David Reasner -S Digitally signed by David Reasner -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=David Reasner -S, 0.9.2342.19200300.100.1.1=2003187028 Date: 2021.09.24 13:05:06 -04'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Product Quality	Hitesh Shroff, PhD	OPQ/ONDP	9 ☒ Authored ☐ Contributed ☐ Approved
Application Technical Lead	**Signature:** Hitesh N. Shroff -S Digitally signed by Hitesh N. Shroff -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000348333, cn=Hitesh N. Shroff -S Date: 2021.09.23 20:48:00 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Charmaine Stewart, MD	OND/DHN	1, 2, 7, 17 and 21 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Charmaine A. Stewart -S Digitally signed by Charmaine A Stewart -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002876488, cn=Charmaine A Stewart -S Date: 2021.09.24 11:26:49 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	George Makar, MD	OND/DHN	All sections ☒ Authored ☒ Contributed ☒ Approved
Cross-Disciplinary Team Lead	**Signature:** George A. Makar -S Digitally signed by George A. Makar -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002928520, cn=George A. Makar -S Date: 2021.09.25 10:34:29 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Regulatory	Lisa Skarupa	OND/ORO	Section 12 ☒ Authored ☐ Contributed ☐ Approved
Project Manager	**Signature:** Lisa M. Skarupa -S Digitally signed by Lisa M. Skarupa -S Date: 2021.09.25 10:10:51 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Regulatory	Ayanna Augustus Bryant	OND/DHN	Section 12 ☐ Authored ☐ Contributed ☒ Approved
CPMS	**Signature:** Ayanna Augustus -S Digitally signed by Ayanna Augustus -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000342669, cn=Ayanna Augustus -S Date: 2021.09.24 12:13:57 -04'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

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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/

GEORGE A MAKAR
09/27/2021 11:44:54 AM

JOSEPH G TOERNER
09/27/2021 12:26:38 PM

JULIE G BEITZ
09/27/2021 12:59:33 PM

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDY

IND/NDA Number:	NDA214662
Drug Name:	Maralixibat chloride
Indication:	Treatment of cholestatic pruritus in patients with Alagille syndrome (ALGS) 1 year of age and older.
Applicant:	Mirum Pharmaceuticals, Inc. 950 Tower Lane, Suite 1050, Foster City, CA 94404
	Test Facility Mice Study: (b) (4) (b) (4)
Documents Reviewed:	Study report (MRXNC-002) submitted on August 31, 2020 via NDA214662/0001 and electronic data submitted on April 7, 2021 via NDA214662/0015
Biometrics Division:	Division of Biometrics -6

Statistical Reviewer:	Zhuang Miao, Ph.D.
Concurring Reviewer:	Karl Lin, Ph.D.
Reviewing Pharmacologist:	Fred Moulin Ph.D.
Keywords:	Carcinogenicity, Dose response

Table of Contents

Summary

In this submission the sponsor included the report of one animal carcinogenicity study in 001178-T (hemizygous) RasH2 mice. This study was intended to assess the carcinogenic potential of Maralixibat, when given daily by oral gavage at appropriate drug levels for 26 weeks in mice.

Mouse Study:

Survival analysis:

1. The survival analyses showed a statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male mice (p-value for likelihood ratio test is 0.0045<0.05, p-value for log-rank test is 0.0156<0.005). The pairwise comparisons showed a statistically positive significant difference in mortality between the vehicle control group and the high dose group in male mice (p-value for likelihood ratio test is 0.0225<0.05).
2. The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in female mice. The pairwise comparisons did not show any statistically positive significant differences in mortality between the vehicle control group and each of the treated groups in female mice.
3. The survival analyses showed statistically positive significant differences in mortality between the vehicle control group and positive control group in both males and females.

Tumor analysis:

1. For male mice, the tumor data analysis showed a statistically significant positive dose response relationship in incidences of the B-ADENOMA, BRONCHIOLO-ALVEO, lung (p-value=0.0036<0.05) and of combined tumors of bronchiola-alveo ADENOMA+CARCINOMA, lung (p-value<0.001) across the vehicle control group and the treated groups. The comparison between the vehicle control and the high dose group showed statistically significant increases in incidences of the B-ADENOMA, BRONCHIOLO-ALVEO, lung (p-value=0.0324<0.05) and of combined tumors of bronchiola-alveo ADENOMA+CARCINOMA, lung (p-value=0.0146<0.05).
2. For female mice, the tumor data analysis showed a statistically significant positive dose response relationship in incidences of the combined tumors of hemangiosarcoma+hemangioma, whole body (p-value=0.0196<0.05) across the vehicle control

group and the treated groups.

3. For male mice, the comparison between the vehicle control and the positive control group showed statistically significant increases in incidence of lymphoma, hemolymphoreticula (p-value<0.001), squamous cell papilloma, skin/subcutis (p-value<0.001), and of squamous cell papilloma, stomach (p-value<0.001).
4. For female mice, the comparison between the vehicle control and the positive control group showed statistically significant increase in incidence of lymphoma, hemolymphoreticula (p-value<0.001), of squamous cell carcinoma, skin/subcutis (p-value=0.0025<0.05), and of squamous cell papilloma, stomach (p-value=0.0025<0.05).

1. Background

In this submission the sponsor included the report of one animal carcinogenicity study in 001178-T (hemizygous) RasH2 mice. This study was intended to assess the carcinogenic potential of Maralixibat,, when given daily by oral gavage at appropriate drug levels for 26 weeks in mice. Results of this review have been discussed with the reviewing pharmacologist Dr. Fred Moulin.

In this review the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as the dose increases.

2. Mouse Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups, one vehicle control group and one positive control group. One hundred 001178-T (hemizygous) RasH2 mice of each sex were randomly assigned to the treated groups and vehicle group in equal size of 25 mice per group. The dose levels for treated groups were 2.5, 7.5 and 25 for males and 7.5, 25 and 75 for females. The mice in the vehicle control group received deionized water. Positive control (N-methyl-N-nitrosourea [MNU]) was administered to the mice in positive control group by intraperitoneal injection on Day 1. There were 10 mice in the positive control group for each sex. The study was designed to continue for up to 26 weeks for both sexes, however in accordance with study termination criteria, all surviving mice were sacrificed during Week 27.

Table 1: Study Design in Mouse Study

Protocol Group No.	Dose Levels (mg/kg/day) Male/Female	Identification	Number of Animals Enrolled	
			Males	Females
1	0	Vehicle	25	25
2	2.5/7.5	Maralixibat	25	25
3	7.5/25	Maralixibat	25	25
4	25/75	Maralixibat	25	25
5	75/75	NMU (Positive Control)	10	10

2.1. Sponsor's analyses

2.1.1. Survival analysis

Data from Groups 1 through 4 were analyzed in accordance with current FDA guidelines (Food and Drug Administration, 2001). Positive control animals (Group 5) were included only to confirm the use of hemizygous animals by an appropriate response (formation of neoplasms) and Group 5 was not included in statistical evaluation.

Tests to compare survival were performed, with a two-sided risk for increasing and decreasing mortality with dose. Tests were performed for dose response and for each dosed group against control using Kaplan-Meier product-limit estimates, along with log-rank and Wilcoxon tests. These were performed using the LIFETEST procedure in SAS. The time to death or sacrifice (in weeks, calculation detailed in the following) was the dependent variable. The test article-treated groups were included as the strata. Animals with a death or sacrifice status recorded as a scheduled sacrifice (interim or terminal) or accidental death were censored in the analysis.

Sponsor’s findings: The sponsor’s analysis showed that the numbers (percents) of survival were 25 (100%), 24 (96%), 25 (100%), and 20 (80%) in male mice, and 25 (100%), 22 (88%), 23 (92%), and 24 (96%), in female mice in vehicol control, low, medium, high dose groups, respectively.

The sponsor concluded that for males, the high-dose group (25 mg/kg/day) had higher mortality than control (5 of 25 versus 0 of 25 in control), with P = 0.0196 for the Log-Rank test and P = 0.0197 for the Wilcoxon test, respectively. The test for trend was also significant, P = 0.0010 for both the Log-Rank and Wilcoxon tests. For females, there were no statistically significant differences in mortality.

2.1.2. Tumor data analysis

Tests to compare tumor incidence were performed with a one-sided risk for increasing incidence with dose. Tests were performed for dose response and for each test article-treated group against the control group.

Unadjusted P-values were reported for tumors. Indication of a possible treatment effect were assessed on the basis of rare or common tumor type, in line with the current FDA guidelines (Food and Drug Administration Draft Guidance for Industry, 2001). The Study Pathologist determined whether a tumor type was rare or common.

Sponsor's findings: The following table presents tumors observed which had statistically significant results in the treated groups when evaluated at the 5% level. Indication of a possible treatment effect was assessed on the basis of rare or common tumor type, in line with the current FDA guidelines (Food and Drug Administration Draft Guidance for Industry, 2001).

Males

Tissue and Lesion	Rare or Common	Test	Unadjusted p-value	FDA Interpretation
Lung B-Adenoma, bronchiolo-alveolar	Common	High v Control	0.0231	Significant ($p<0.05$)
Lung B-Adenoma, bronchiolo-alveolar	Common	Trend	0.0024	Significant ($p<0.01$)
Lung B-Adenoma, bronchiolo-alveolar / M-Carcinoma, bronchiolo-alveolar	Common	High v Control	0.0096	Significant ($p<0.05$)
Lung B-Adenoma, bronchiolo-alveolar / M-Carcinoma, bronchiolo-alveolar	Common	Trend	0.0006	Significant ($p<0.01$)

Females

Tissue and Lesion	Rare or Common	Test	Unadjusted p-value	FDA Interpretation
Ovary, Spleen, Uterus B-Hemangioma / M-Hemangiosarcoma	Common	Trend	0.0187	Not significant ($p\geq0.01$)

2.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses.

2.2.1. Survival analysis

The survival distributions of three treated groups, one vehical control group, and one positive control group were estimated using the Kaplan-Meier product limit method. The dose response relationship in survival was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 1 and 2 in the appendix for male and female mice, respectively. The intercurrent mortality data are given in Tables 6 and 7 in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals among the vehicle control and three treated groups are given in Tables 8 and 9 in the appendix for male and female mice, respectively.

Reviewer's findings: This reviewer's analysis showed that the numbers (percents) of survival were 25 (100%), 24 (96%), 25 (100%), 20 (80%) and 0 (0%) in male mice, and 25 (100%), 22 (88%), 23 (92%), 24 (96%) and 2 (20%) in female mice in vehicol control, low, medium, high dose groups and positive control group, respectively.

The survival analyses showed a statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male mice (p-value for likelihood ratio test is 0.0045<0.05, p-value for log-rank test is 0.0156<0.005). The pairwise comparisons showed a statistically positive significant difference in mortality between the vehicle control group and the high dose group in male mice (p-value for likelihood ratio test is 0.0225<0.05).

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in female mice. The pairwise comparisons did not show any statistically positive significant differences in mortality between the vehicle control group and each of the treated groups in female mice.

The survival analyses showed statistically positive significant differences in mortality between the vehicle control group and positive control group in both males and females.

2.2.2. Tumor data analysis

The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons between vehicle control and three treated groups, between saline control and the treated groups and between vehicle control and positive control are listed in Tables 10, 11, 12 and 13 in the appendix for male and female mice, respectively.

Adjustment for multiple testing: For the adjustment of multiple testing of dose response relationship for the transgenic mouse study in a submission with only one transgenic mouse study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels α =0.05 for both common tumors and rare tumors for the mouse study. For pairwise, the same guidance document suggests the use of test levels α =0.05 for both common tumors and rare tumors for the mouse study.
Reviewer's findings: The tumor types in Tables 2, 3, 4 and 5 below showed p-values less than or equal to 0.05 in the tests for pairwise comparisons between vehicle and the positive control group for male mice and female mice, respectively.

Table 2: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	2.5 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	7.5 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	25 mg/kg/day High (N=25) P-value - Vehicle vs. High
LUNG	B-ADENOMA, BRONCHIOLO-ALVEO*	1/25 (25) 0.0036	2/25 (25) 0.5000	0/25 (25) 1.0000	6/25 (22) 0.0324
	C_bronchiola-alveo ADENOMA+CARCINOMA	1/25 (25) <0.001	2/25 (25) 0.5000	0/25 (25) 1.0000	7/25 (22) 0.0146

& 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 3: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Vehicle Control and Treated Groups-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	7.5 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	25 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	75 mg/kg/day High (N=25) P-value - Vehicle vs. High
Whold Body	C_Hemangiosarcoma+Hemangioma	0/25 (25) 0.0196	0/25 (23) NC	1/25 (24) 0.4898	3/26 (25) 0.1173

Table 4: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Vehicle Control and Positive Control-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
HEMOLYMPHO- RETICU	M-MALIGNANT LYMPHOMA	0/25 (25)	8/10 (9) <0.001
SKIN/SUBCUTIS	B-PAPILLOMA, SQUAMOUS CELL	0/25 (25)	6/10 (7) <0.001
STOMACH, NONGLANDU	B-PAPILLOMA, SQUAMOUS CELL	0/25 (25)	7/10 (8) <0.001

Table 5: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Vehicle Control and Positive Control-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
HEMOLYMPHO- RETICU	M-MALIGNANT LYMPHOMA	0/25 (25)	9/10 (9) <0.001
SKIN/SUBCUTIS	M-CARCINOMA, SQUAMOUS CELL	0/25 (25)	3/10 (5) 0.0025
STOMACH, NONGLANDU	B-PAPILLOMA, SQUAMOUS CELL	0/25 (25)	3/10 (5) 0.0025

Reviewer's findings: Based on the criteria of adjustment for multiple testing discussed in the mouse study data analysis section, we make the following statistical conclusions:

1. For male mice, the tumor data analysis showed a statistically significant positive dose response relationship in incidences of the B-ADENOMA, BRONCHIOLO-ALVEO, lung (p-value=0.0036<0.05) and of combined tumors of bronchiola-alveo ADENOMA+CARCINOMA, lung (p-value<0.001) across the vehicle control group and the treated groups. The comparison between the vehicle control and the high dose group showed statistically significant increases in incidences of the B-ADENOMA, BRONCHIOLO-ALVEO, lung (p-value=0.0324<0.05) and of combined tumors of bronchiola-alveo ADENOMA+CARCINOMA, lung (p-value=0.0146<0.05).
2. For female mice, the tumor data analysis showed a statistically significant positive dose response relationship in incidences of the combined tumors of hemangiosarcoma+hemangioma, whole body (p-value=0.0196<0.05) across the vehicle control group and the treated groups.
3. For male mice, the comparison between the vehicle control and the positive control group showed statistically significant increases in incidence of lymphoma, hemolymphoreticula (p-value<0.001), of squamous cell papilloma, skin/subcutis (p-value<0.001), and of squamous cell papilloma, stomach (p-value<0.001).
4. For female mice, the comparison between the vehicle control and the positive control group showed statistically significant increase in incidence of lymphoma, hemolymphoreticula (p-value<0.001), of squamous cell carcinoma, skin/subcutis (p-value=0.0025<0.05), and of squamous cell papilloma, stomach (p-value=0.0025<0.05).

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Mathematical
Statistician
Concur:
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cc:
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3. Appendix

Table 6: Intercurrent Mortality Rate -Male Mice

	Vehicle 0 mg|kg|day		2.5 mg|kg|day		7.5 mg|kg|day		25 mg|kg|day		Positive 75 mg|kg|day	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 13	.	.	.	.	.	.	2	8.00	1	10.00
14 - 26	.	.	1	4.00	.	.	3	12.00	9	90.00
Ter. Sac.	25	100.00	24	96.00	25	100.00	20	80.00	.	.

Cum. %: Cumulative percentage except for Ter. Sac.

Table 7: Intercurrent Mortality Rate -Female Mice

	Vehicle 0 mg|kg|day		7.5 mg|kg|day		25 mg|kg|day		75 mg|kg|day		Positive 75 mg|kg|day	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 13	.	.	1	4.00	.	.	.	.	3	30.00
14 - 26	.	.	2	8.00	2	8.00	1	4.00	5	50.00
Ter. Sac.	25	100.00	22	88.00	23	92.00	24	96.00	2	20.00

Cum. %: Cumulative percentage except for Ter. Sac.

Table 8: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control, Positive Control and Vehicle Control-Male Mice

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Dose-Response	Likelihood Ratio	0.0045	0.3166	.	0.0225	<0.0001
Homogeneity	Log-Rank	0.0156	0.4197	.	0.0645	<0.0001

Table 9: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control, Positive Control and Vehicle Control -Female Mice

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Dose-Response	Likelihood Ratio	0.8284	0.0800	0.1548	0.3166	<0.0001
Homogeneity	Log-Rank	0.5914	0.1572	0.2510	0.4197	<0.0001

Table 10: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	2.5 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	7.5 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	25 mg/kg/day High (N=25) P-value - Vehicle vs. High
HARDERIAN GLAND	B-ADENOMA	0/25 (25) 0.7423	1/25 (25) 0.5000	0/25 (25) NC	0/25 (22) NC
KIDNEY	B-HEMANGIOMA	0/25 (25) 0.4845	0/25 (25) NC	1/25 (25) 0.5000	0/25 (22) NC
LUNG	B-ADENOMA, BRONCHIOLO-ALVEO*	1/25 (25) 0.0036	2/25 (25) 0.5000	0/25 (25) 1.0000	6/25 (22) 0.0324
	C_bronchiola-alveo ADENOMA+CARCINOMA	1/25 (25) <0.001	2/25 (25) 0.5000	0/25 (25) 1.0000	7/25 (22) 0.0146
	M-CARCINOMA, BRONCHIOLO-ALV*	0/25 (25) 0.2268	0/25 (25) NC	0/25 (25) NC	1/25 (22) 0.4681
SKIN/SUBCUTIS	M-HEMANGIOSARCOMA	0/25 (25) 0.2268	0/25 (25) NC	0/25 (25) NC	1/25 (22) 0.4681
SPLEEN	M-HEMANGIOSARCOMA	0/25 (25) 0.2763	1/25 (25) 0.5000	1/25 (25) 0.5000	1/25 (23) 0.4792
THYMUS	B-THYMOMA	2/24 (24) 0.9844	1/25 (25) 0.8901	0/23 (23) 1.0000	0/24 (21) 1.0000
Whold Body	C_Hemangiosarcoma+Hemangioma	0/25 (25) 0.1192	1/25 (25) 0.5000	2/25 (25) 0.2449	2/25 (23) 0.2243

Table 11: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	7.5 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	25 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	75 mg/kg/day High (N=25) P-value - Vehicle vs. High
HARDERIAN GLAND	B-ADENOMA	2/25 (25) 0.6895	1/25 (23) 0.8670	0/25 (24) 1.0000	1/26 (25) 0.8827
HEMOLYMPHO-RETICU	M-MALIGNANT LYMPHOMA	0/25 (25) 0.7423	1/25 (23) 0.4792	0/25 (24) NC	0/26 (25) NC
LUNG	B-ADENOMA, BRONCHIOLO-ALVEO*	0/25 (25) 0.6289	1/25 (23) 0.4792	1/25 (24) 0.4898	0/26 (25) NC
OVARY	B-HEMANGIOMA	0/25 (25) 0.2577	0/25 (23) NC	0/25 (24) NC	1/26 (25) 0.5000
SPLEEN	B-HEMANGIOMA	0/25 (25) 0.5052	0/25 (23) NC	1/25 (24) 0.4898	0/26 (25) NC
	M-HEMANGIOSARCOMA	0/25 (25) 0.2577	0/25 (23) NC	0/25 (24) NC	1/26 (25) 0.5000
STOMACH, NONGLANDU	B-PAPILLOMA, SQUAMOUS CELL	0/25 (25) 0.5052	0/25 (23) NC	1/25 (24) 0.4898	0/26 (25) NC
UTERUS	B-HEMANGIOMA	0/25 (25) 0.2577	0/25 (23) NC	0/25 (24) NC	1/26 (25) 0.5000
Whold Body	C_Hemangiosarcoma+Hemangioma	0/25 (25) 0.0196	0/25 (23) NC	1/25 (24) 0.4898	3/26 (25) 0.1173

Table 12: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control -Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=10) P-value - Vehicle vs. Positive
HEMOLYMPHO-RETICU	M-MALIGNANT LYMPHOMA	0/25 (25)	8/10 (9) <0.001
LUNG	B-ADENOMA, BRONCHIOLO-ALVEO*	1/25 (25)	0/10 (4) 1.0000
	C_bronchiola-alveo ADENOMA+CARCINOMA	1/25 (25)	0/10 (4) 1.0000
SKIN/SUBCUTIS	B-PAPILLOMA, SQUAMOUS CELL	0/25 (25)	6/10 (7) <0.001
	M-CARCINOMA, SQUAMOUS CELL	0/25 (25)	1/10 (5) 0.1667
STOMACH, NONGLANDU	B-PAPILLOMA, SQUAMOUS CELL	0/25 (25)	7/10 (8) <0.001
	M-CARCINOMA, SQUAMOUS CELL	0/25 (25)	1/10 (5) 0.1667
TESTIS	B-INTERSTITIAL CELL TUMOR	0/25 (25)	1/10 (4) 0.1379
THYMUS	B-THYMOMA	2/24 (24)	0/10 (4) 1.0000

Table 13: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control -Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=10) P-value - Vehicle vs. Positive
HARDERIAN GLAND	B-ADENOMA	2/25 (25)	0/10 (4) 1.0000
HEMOLYMPHO-RETICU	M-MALIGNANT LYMPHOMA	0/25 (25)	9/10 (9) <0.001
SKIN/SUBCUTIS	M-CARCINOMA, SQUAMOUS CELL	0/25 (25)	3/10 (5) 0.0025
STOMACH, NONGLANDU	B-PAPILLOMA, SQUAMOUS CELL	0/25 (25)	3/10 (5) 0.0025
UTERUS	B-HEMANGIOMA	0/25 (25)	1/10 (5) 0.1667
Whold Body	C_Hemangiosarcoma+Hemangioma	0/25 (25)	1/10 (5) 0.1667

Figure 1: Kaplan-Meier Survival Functions for Male Mice

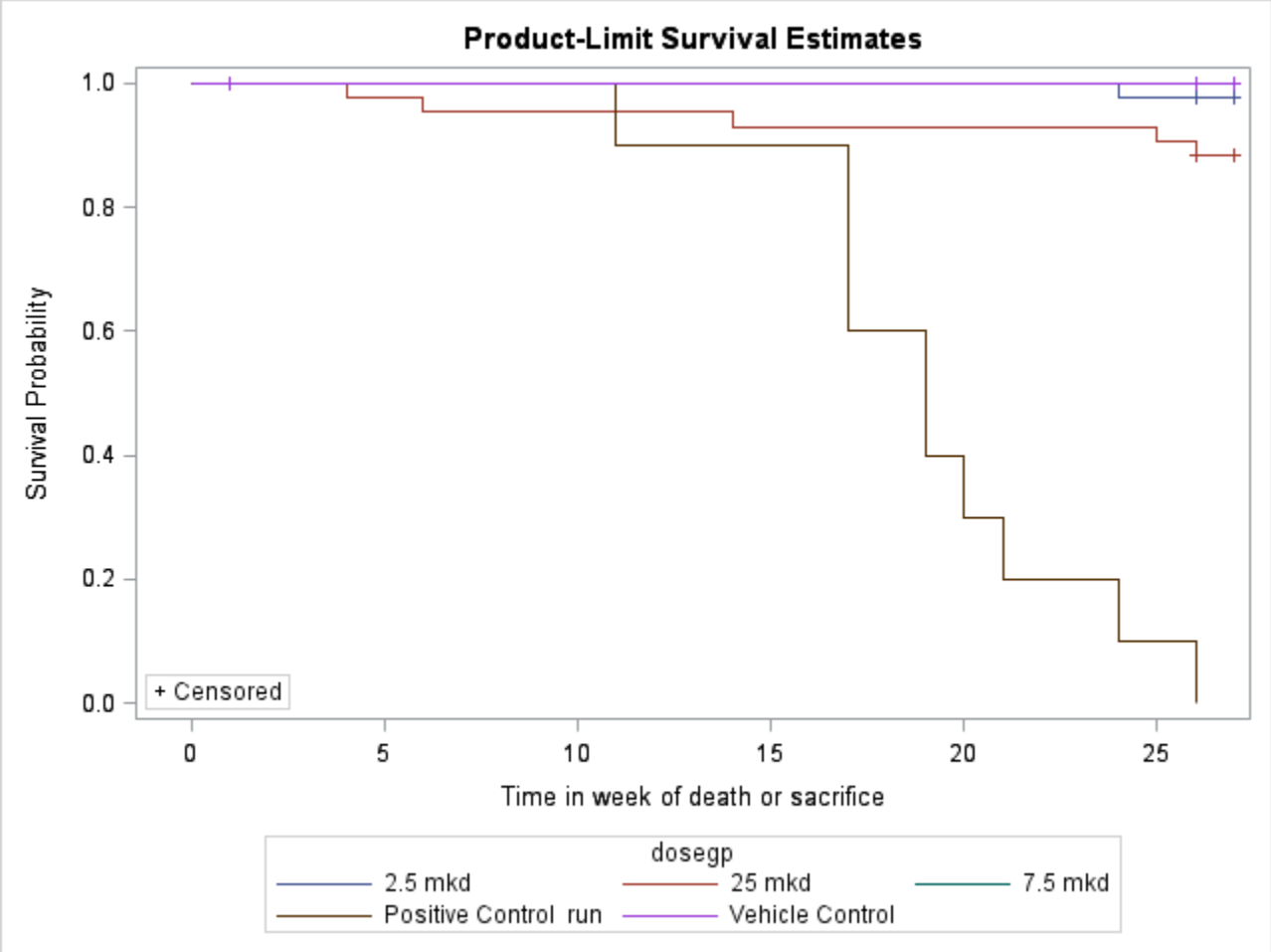
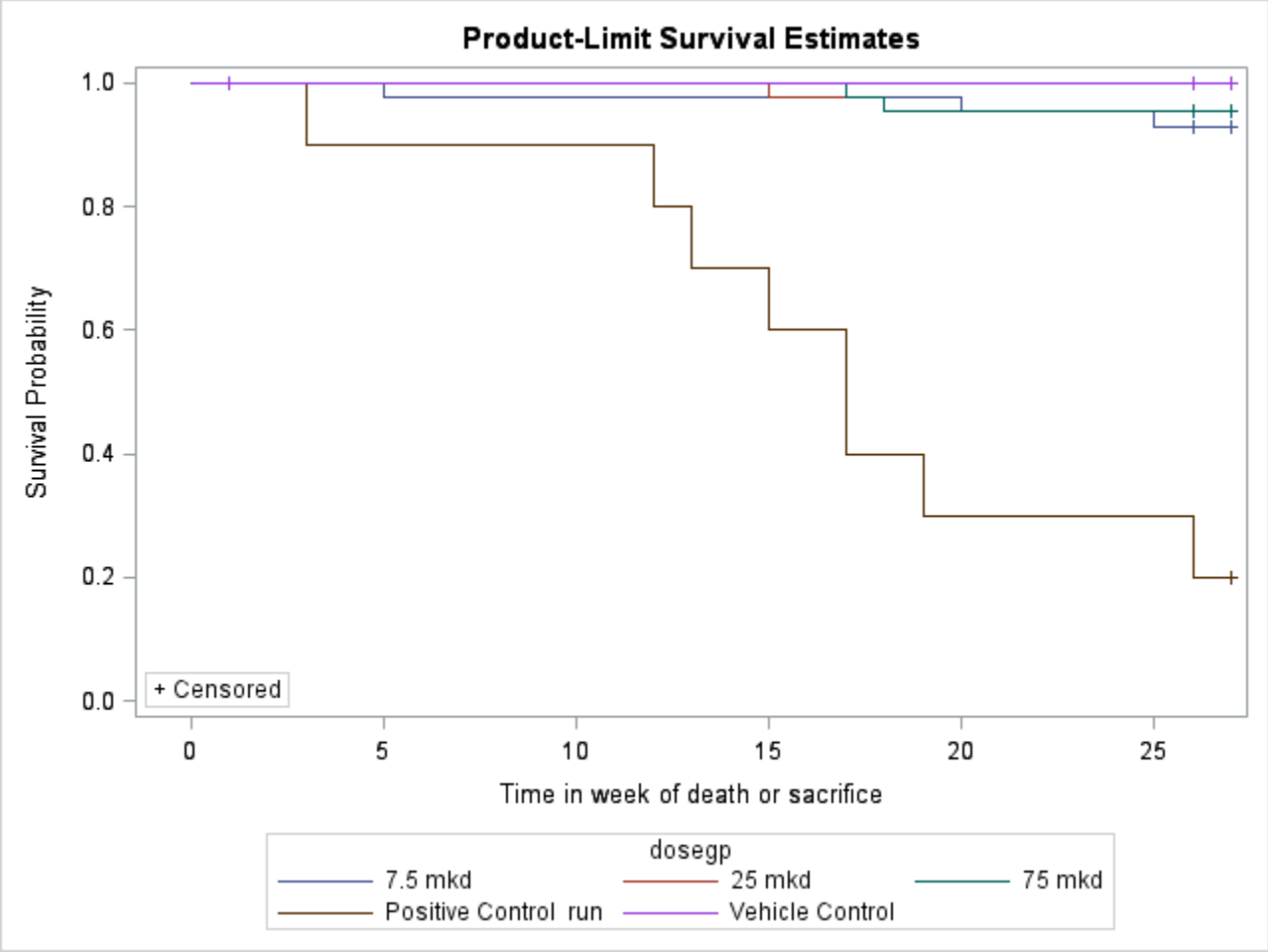


Figure 2: Kaplan-Meier Survival Functions for Female Mice



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