25 March 2021
EMA/574349/2021
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Livmarli

International non-proprietary name: maralixibat

Procedure No. EMEA/H/C/005551/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AES	atomic emission spectroscopy
AET	Antimicrobial effectiveness Test
ALGS	Alagille Syndrome
API	Active Pharmaceutical Ingredient
AR	assessment report
ASM	Active Substance Manufacturer
ASMF	Active Substance Master File = Drug Master File
AQL	Acceptable Quality Limit
AUC	area under the curve
BDL	Below the limit of detection
BA	Bile acid
BSEP	Bile salt export pump
CCS	Container Closure System
CEP	Certificate of Suitability of the EP
CMS	Concerned Member State
CTD	common technical document
CoA	Certificate of Analysis
CQAs	Critical Quality Attributes
CRS	Chemical reference substance
DL	Detection Limit
DMF	Dimethylformamide
DOM	Date of manufacture
DSC	Differential scanning Calorimetry
ECD	Electrochemical detection
EDMF	European Drug Master File
EDQM	European Directorate for the Quality of Medicines
ee	enantiomeric excess
EP	European Pharmacopoeia
FDA	US Food and Drug Administration
FDSC	Fixed Drug Substance Concentration
FDV	Fixed Dosing Volume
FID	Flame ionisation detection
FPM	finished product manufacturer
FTIR	Fourier transmission infrared (spectroscopy)
GC	Gas chromatography
GMP	good manufacturing practice
HCC	Hepatocellular carcinoma
HDPE	high density polyethylene
HPLC	High performance liquid chromatography
ICH	International conference on harmonization
ICP	Inductively coupled plasma
IPA	isopropyl alcohol
IPC	In-process control test
IR	Infra-red
IRC	immediate release capsules
KF	Karl Fischer
LoA	Letter of Access
LOD	Loss on Drying
LoD	Limit of detection
LoQ	Limit of Quantitation
MA	Marketing Authorisation
MAH	Marketing Authorisation holder
MCC	microcrystalline cellulose
MS	Mass spectroscopy
NfG	Note for Guidance
NIR	Near Infra Red
NLT	Not less than

NMR	Nuclear magnetic resonance
NMT	Not more than
PBC	Primary Biliary Cirrhosis
PCTFE	Polychlorotrifluoroethene
PDA	Photo diode array
PDE	Permitted Daily Exposure
PFIC	Progressive Familial Intrahepatic Cholestasis
PPQ	Process performance qualification
PSC	Primary Sclerosing Cholangitis
PVC	Polyvinyl chloride
PVdC	Polyvinyl dichloride
Ph.Eur.	European Pharmacopoeia
QL	Quantitation limit
QOS	Quality Overall Summary
QTPP	Quality Target Product Profile
RH	Relative Humidity
RMS	Reference member state
ROI	residue on ignition
RRt	Relative retention time
Rt	Retention time
RT	Room temperature
SAL	Sterility assurance level
SEM	Scanning electron microscopy
SR	sustained release tablets
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TGA	Thermo-Gravimetric Analysis
UV	Ultra violet
XRD	X-Ray Diffraction
XRPD	X-Ray PowderDiffraction

N.B. → Not all abbreviations are used in this report

1. CHMP Recommendations

Based on the review of the data on quality, safety, efficacy, the application for maralixibat (LIVMARLI®), 9.5 mg/ml oral solution, an orphan medicinal product

for the treatment of Progressive Familial Intrahepatic Cholestasis Type 2 (PFIC2) in patients 1 year of age and older,

is **not approvable**

since **"major objections"** have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the List of Questions (see section VI). In addition, satisfactory answers must be given to the "other concerns" as also detailed in the List of Questions below.

The major objections precluding a recommendation of marketing authorisation, pertain to the following principal deficiencies:

Quality:

Redefinition of the designated starting material is necessary because the starting material consists of a complex ring structure including formation of chiral centers which is considered critical and should be covered by GMP.

Since the drug product is packaged in a semi-permeable container, the potential water loss should be evaluated under conditions of low relative humidity as stipulated in the guideline ICH Q1A / Stability testing of new drug substances and products.

Clinical:

The submitted clinical program is considered insufficient to support the targeted indication. Firm conclusions on efficacy of maralixibat in the PFIC2 patient population cannot be drawn at this point in time based on the available data. Therefore, benefit-risk assessment is negative.

Studied population is not supportive of the target population and justification of the choice of broader indication is necessary.

Questions to be posed to additional experts

Currently no questions proposed.

Inspection issues

GMP inspection(s)

Currently no GMP inspection proposed.

GCP inspection(s)

Decision whether or not a GCP inspection is necessary will be taken later.

New active substance status

Based on the review of the data the active substance maralixibat chloride contained in the medicinal product Livmarli is considered to be qualified as a new active substance in itself.

Additional data exclusivity /Marketing protection

N/A

Similarity with authorised orphan medicinal products

N/A. There is no authorised product for the proposed indication PFIC2.

Derogation(s) from market exclusivity

N/A

2. Executive summary

2.1. Problem statement

2.1.1. Disease or condition

Maralixibat is being developed for the treatment of Progressive Familial Intrahepatic Cholestasis Type 2 (PFIC2) in patients 1 year of age and older.

PFIC refers to a heterogeneous group of inherited autosomal recessive liver disorders characterized by intrahepatic cholestasis due to defects in bile acid transportation from hepatocytes to bile canaliculi (Davit-Spraul et al. 2009; Mehl et al. 2016).

2.1.2. Epidemiology and risk factors, screening tools/prevention

The exact prevalence of PFIC is unknown, but the disease is estimated to affect 1 in every 50,000 to 100,000 births (Davit-Spraul et al. 2009). PFIC represents 10%–15% of causes of cholestasis in children (Gunaydin and Bozkurter Cil 2018) and 10%–15% of indications for liver transplantations in children (Davit-Spraul et al. 2009).

2.1.3. Biologic features, Aetiology and pathogenesis

The main subtypes PFIC1, PFIC2, and PFIC3 are caused by mutations in ATP8B1, ABCB11, and ABCB4, respectively (Amer and Hajira 2014). In addition, more rare subtypes of PFIC (TJP2, NR1H4, and MYO5B mutations) have recently been identified.

- PFIC1 (also referred to as Byler disease), caused by mutations in ATP8B1 (a phospholipid transporting floppase expressed in the liver), comprises approximately 10%–20% of the PFIC population (Alissa et al. 2008; Davit-Spraul et al. 2009).

- PFIC2, or BSEP deficiency, is caused by mutations in ABCB11, this mutation is predominate and PFIC2 is the most common PFIC subtype. (50-60%)

- PFIC3, caused by mutations in ABCB4 (a transporter involved in phospholipid excretion in the liver), occurs in approximately 30%–40% of the PFIC population.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

All PFIC subtypes share the main clinical manifestations of cholestasis and are associated with early mortality, morbidity, and severe pruritus with devastating consequences on patients' QoL. As a result of

impaired bile flow, sBA levels in PFIC are severely and persistently elevated (Morris et al. 2015). The accumulation of bile acids results in progressive liver damage, and if left untreated, can lead to end-stage liver disease and death (Mehl et al. 2016; Bull and Thompson 2018). Due to a lack of treatment options, 70%–80% of patients ultimately require liver transplantation.

Children often present with jaundice and debilitating pruritus within the first years of life. The pruritus is often so distressing that it often requires surgical therapy. It often leads to sleep disturbances, fatigue, irritability, cutaneous self-mutilation, poor attention and school performance, as well as impaired QoL. Many patients will go on to receive a liver transplant in the absence of liver failure to provide relief from pruritus (Bull and Thompson 2018). In addition to pruritus, other disease manifestations include growth retardation, fat malabsorption, deficiencies in FSVs, and elevated transaminases and bilirubin (Srivastava 2014; Mehl et al. 2016). PFIC has also been associated with the development of hepatocellular carcinoma (HCC) at an early age.

On average, patients with PFIC2 require transplants earlier than those with other PFIC subtypes. Approximately only 45% and 32% of all patients with PFIC2 will survive with their native liver to Year 10 and Year 18, respectively (van Wessel et al. 2020).

2.1.5. Management

The only definitive treatment currently available for PFIC is liver transplantation.

Currently, there are no approved pharmacological therapies to treat symptoms or the underlying liver disease of PFIC. According to the European Association for the Study of the Liver (EASL 2009), "no medical therapy of proven benefit for the long-term prognosis of PFIC exists." In the absence of a curative treatment option, Surgical Biliary Diversion Procedures (SBD) and liver transplantation are commonly employed to address symptoms and improve QoL as well as the underlying liver disease.

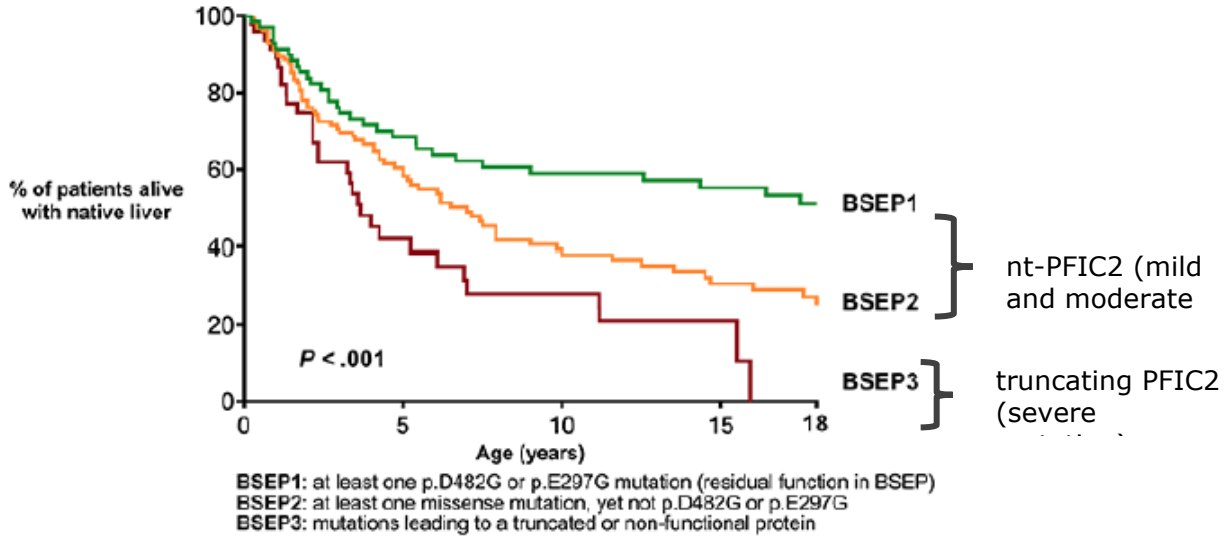
Given the lack of approved pharmacotherapy, the invasive nature of surgical treatment options and patients' short- and long-term morbidity and mortality, it is acknowledged that there remains a high unmet medical need for an easily reversible pharmacological treatment alternative in patients with PFIC that is safe and efficacious in prolonging their liver survival and easing disease burden.

Pharmacologic interruption of the enterohepatic circulation, through inhibition of the ASBT with a minimally absorbed compound such as maralixibat, might be an attractive alternative to SBD and liver transplantation in this patient population, provided it is clinically relevant efficacious and has a reliably measurable impact on the symptoms in terms of QoL.

<u>Surgical Interventions</u>

Surgical interventions to treat the pruritus and cholestasis associated with PFIC2 include surgical biliary diversion procedures (SBD) procedures and liver transplantation. All patients with PFIC2, regardless of mutational status, are at risk of liver disease progression and may eventually need liver transplantation (van Wessel et al. 2020). In patients with PFIC2, only around 45% and 32% will survive with their native liver to Year 10 and Year 18, respectively (Figure 1; van Wessel et al. 2020).

Figure 1 Percent of Patients with Native Liver Survival by Genotype Severity



BSEP = bile salt export pump; PFIC = Progressive Familial Intrahepatic Cholestasis.

Note: Nontruncating mild mutation = BSEP1; Nontruncating moderate mutation = BSEP2; truncating severe mutation = BSEP3.
Source: van Wessel et al. 2020.

There is a considerable unmet medical need for both definitive treatments and symptomatic treatments of PFIC2

Liver transplantation is currently the only definitive treatment available for PFIC, although disease recurrence and early graft failure are not uncommon. Liver transplantation is indicated in PFIC to address both severe pruritus as well as progressive liver damage caused by bile acid accumulation (Mehl et al 2016; Bull and Thompson 2018). It corrects the underlying bile acid excretion defect and remains the only treatment option for end-stage liver disease in PFIC (EASL 2009; Mehl et al. 2016).

2.2. About the product

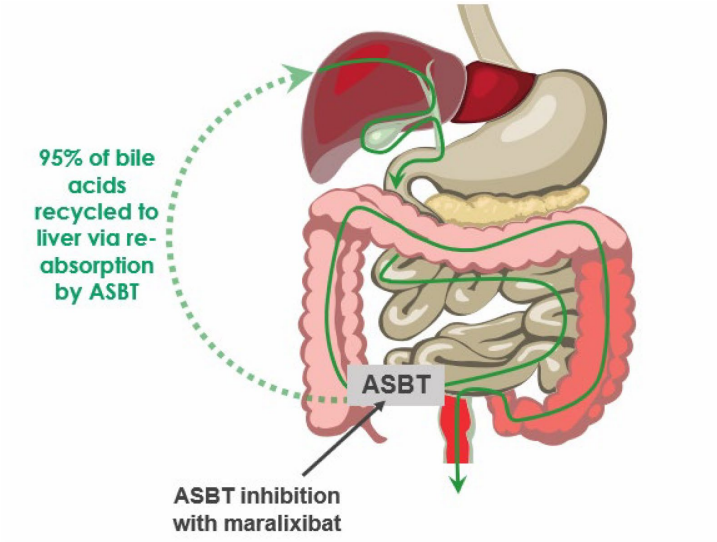
Mode of action: Maralixibat chloride (formerly known as SD-5613, SHP625, and LUM001; hereafter referred to as maralixibat) is an inhibitor of the ASBT.

This transmembrane protein transporter, localized on the luminal surface of ileal enterocytes, is present in the terminal 25% of the small intestine and mediates uptake of conjugated bile acids across the brush border membrane of the enterocyte.

Maralixibat is a potent, highly selective ASBT inhibitor (IC50 = 0.3 nM) as demonstrated in cell-based assays. Maralixibat is minimally absorbed due to its large molecular weight (710 Da) and the presence of a positively charged quaternary nitrogen atom, therefore maximizing the local exposure of the molecule to its target and minimizing unnecessary systemic exposure.

Maralixibat-mediated blockade of intestinal reabsorption of bile acids by ASBT interrupts the enterohepatic circulation, thereby increasing fBA excretion and lowering sBA levels (Figure 2).

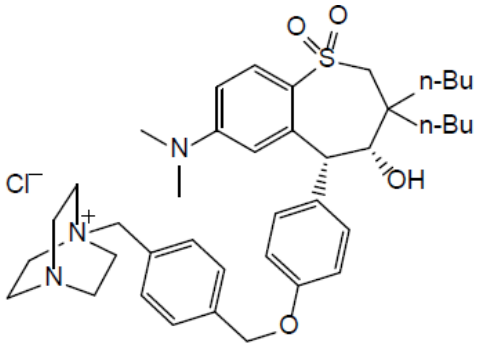
Figure 2 Interruption of Enterohepatic Circulation of Bile Acids by Maralixibat



ASBT = apical sodium-dependent bile acid transporter.

Pharmacological classification

Figure 3 Structure of MRX Drug Substance



Chemical Name(s)

Maralixibat chloride: (4R,5R)-1-[[4-[[4-[3,3-Dibutyl-7- (dimethylamino)-2,3,4,5-tetrahydro-4-hydroxy-1,1-dioxido-1- benzothiepin-5-yl] phenoxy] methyl] phenyl] methyl]-4-aza-1-azoniabicyclo[2.2.2]octane chloride

Maralixibat: (4R,5R)-1-[[4-[[4-[3,3-Dibutyl-7-(dimethylamino)-2,3,4,5-tetrahydro-4-hydroxy-1,1-dioxido-1-benzothiepin-5-yl] phenoxy] methyl] phenyl] methyl]-4-aza-1-azoniabicyclo[2.2.2]octane

Chemical Abstracts Service (CAS) Registry Number:

Maralixibat Chloride**:** 228113-66-4

Maralixibat: 716313-53-0

The proposed commercial formulation is an oral solution that will be measured for dosing on a weight basis and be taken once or twice daily. It is supplied as a 9.5 mg/mL ready-to-use fixed concentration of maralixibat free base solution (equivalent to 10 mg/mL maralixibat chloride).

The proposed commercial dosing regimen is 266 µg/kg maralixibat free base (equivalent to 280 µg/kg maralixibat chloride) once daily, taken approximately 30 minutes before the first meal of the day. Dose may be increased to 266 µg/kg twice daily with the second daily dose taken approximately 30 minutes before the evening meal.

2.3. The development programme/compliance with CHMP guidance/scientific advice

Maralixibat was originally developed as a lipid-lowering agent (Table 3). It was subsequently developed for the treatment of cholestatic diseases by Lumena Pharmaceuticals, Inc. and Maralixibat Chloride Mirum Pharmaceuticals, Inc. Shire Human Genetic Therapies, Inc. Ownership of the maralixibat development program was transferred to Mirum Pharmaceuticals, Inc. in December 2018. Maralixibat was previously studied in healthy volunteers as well as adults and adolescents with elevated cholesterol, and adults with Primary Biliary Cholangitis (PBC), and Primary Sclerosing Cholangitis (PSC). Indications of hypercholestrolemia, PSC and PBC are no longer being pursued.

Table 1 Development and Sponsorship for Maralixibat

Date	Sponsors	Targeted Diseases
15 June 1999	G.D. Seale & Co.	Primary hypercholesterolemia
5 June 2003	Pharmacia & Upjohn Company	
15 July 2005	Pfizer Global Research & Development	
26 April 2013 to February 2015	Lumena Pharmaceuticals, Inc.	Cholestatic diseases (ALGS, PFIC, PBC, and PSC)
February 2015 to 17 December 2018	Shire Human Genetic Therapies, Inc.	
18 December 2018	Mirum Pharmaceuticals, Inc.	
7 August 2020	Mirum Pharmaceuticals, Inc.	BA

ALGS = Alagille syndrome; BA = biliary atresia; IND = Investigational New Drug; PBC = primary biliary cholangitis; PFIC = progressive familial intrahepatic cholestasis; PSC = primary sclerosing cholangitis.

The applicant is currently developing maralixibat for the treatment of ALGS, PFIC, and biliary atresia. The development program of maralixibat was discussed at two Protocol Assistance meetings with the EMA.

The key regulatory advice provided by EMA supporting the development of maralixibat in PFIC and highlighted the following issues:

- the EMA agreed that there is an unmet medical need for the development of new treatment strategies for PFIC2.
- In regard to clinical efficacy, the Study LUM001-501 results and proposed Phase 3 Study MRX-502 were discussed, and it was agreed that the endpoints employed in these studies and the amount of data from Study LUM001-501 at the time would support an indication on symptomatic relief for the "treatment of pruritus associated with PFIC."
- The design of Study MRX-502 was intended to be a confirmatory trial for Study LUM001-501; thus, Study MRX-502 is being conducted as a 26-week, double-blind, placebo-controlled study with a primary objective to evaluate the efficacy of maralixibat on reducing the severity of pruritus. However, the CHMP also emphasized "that for an indication of 'treatment of PFIC', even if limited to a subset (i.e., PFIC2), support/demonstration of clear benefit in terms of clinically relevant hard endpoints such as postponement or deferral of liver transplant would be ultimately required."

However, obviously the applicant decided later to seek approval on base of historical controls only.: *With the recent availability of natural history data from the NAPPED study (van Wessel et al. 2020), Mirum is submitting this MAA seeking full approval, providing data from participants with PFIC2 from the proposed pivotal long-term maralixibat Study LUM001-501.*

Additional advice provided by the Rapporteur (BfArM) and the Co-Rapporteur (DKMA) at the presubmission meetings.

2.4. General comments on compliance with GMP, GLP, GCP

GMP: Valid GMP certificates are provided for the API manufacturer, and the genotoxic testing facility. The drug product manufacturer has been inspected by Health Canada and FDA. A QP statement has been provided issued by the site responsible for batch release and based on an audit.

GLP: Regarding pharmacology, the in-vitro hERG study and one of the in-vivo primary pharmacodynamics studies were performed compliant with GLP regulations.
The Single dose toxicity studies performed in the rat (with oral and IV administration) were conducted compliant to GLP regulations, as were an oral single dose study and a single dose study with IV administration in the dog.

The pivotal repeated dose toxicity and carcinogenicity studies in mice, rats, dogs and genotoxicity evaluations were performed according to the study reports compliant to GLP regulations.

Pivotal non-clinical studies were conducted at several different facilities or CROs. A vast majority of the repeat-dose toxicity studies, especially in dogs, were conducted . Additionally, a number of repeat-dose studies in rats were conducted in combination with testing preformed.

Pivotal reproductive and developmental toxicity studies were to a large degree conducted.

Newer studies (e.g. a 13-week repeat-dose rat study and the juvenile toxicity studies) were conducted.

GLP compliance were stated for all pivotal studies. GLP compliance was confirmed by FDA in the period of the conducted studies. However, GLP compliance could not be confirmed, which is relevant in context to two repeat-dose studies in rats (i.e. SA5006/MSE-N0003/R7248-SHP625 and SA4988/ MSE-N 99078/SA4988). The Applicant, is therefore asked to provided certificates to support compliance with OECD/MAD GLP and/or selected inspection reports covering the time of study conduct **(OC)**.

GCP: The applicant declared that all clinical studies in this application were undertaken in accordance with the principles of GCP. All studies were conducted with the approval of Ethics Committees or Institutional Review Boards.

Informed consent was obtained for all participants, and the studies were performed in accordance with the version of the Declaration of Helsinki that applied at the time the studies were conducted. Where required, regulatory approval was obtained from the relevant health authority.

There are, however, currently concerns raised that in the pivotal single study conducted in the targeted population (LUM001-501) GCP rules might have been breached. Additional information is requested. Decision on the necessity of GCP inspection will be taken after the additional information has been evaluated.

2.5. Type of application and other comments on the submitted dossier

Legal basis

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application.

PRIME

Not applied

Accelerated assessment

Not applied

Conditional marketing authorisation

Not applied.

Marketing authorisation under exceptional circumstances

Not applied

Biosimilarity

N/A

Additional data exclusivity/ marketing protection

N/A

New active substance status

The applicant **requested** the active substance maralixibat chloride contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Orphan designation

Maralixibat [(4R,5R)-1-[[4-[[4-[3,3-dibutyl-7-(dimethylamino)-2,3,4,5-tetrahydro-4-hydroxy-1,1-dioxido-1-benzothiepin-5-yl]phenoxy]methyl]phenyl]methyl]-4-aza-1-azoniabicyclo[2.2.2]octane chloride9] **was designated as an orphan medicinal product** EU/3/13/1216 on 18.12.2013 in the following condition: Treatment of progressive familial intrahepatic cholestasis.

Similarity with orphan medicinal products

The application did currently not contain a critical report pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, addressing the possible similarity with authorised orphan medicinal products. There is currently no product licensed for the indication proposed (PFIC2).

Derogation(s) from orphan market exclusivity

N/A

Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0200/2020 on the agreement of a paediatric investigation plan (PIP).

A Paediatric Investigational Plan (PIP) for maralixibat chloride for the treatment of PFIC was approved by the EMA on 30 January 2018 (EMEA-001475-PIP03-17). The EMA decision (P/0024/2018), including all annexes, issued on 30 January 2018 is enclosed in the submission (001475-PIP03-17 Decision with annexes). A first modification of this PIP was agreed on 6 December 2019 (EMEA-001475-PIP03-17-M01). The EMA decision (P/0409/2019), including all annexes, issued on 6 December 2019 is enclosed (001475-PIP03-17-M01 Decision with annexes). A second modification of this PIP was agreed on 25 May 2020 (EMEA-001475-PIP03-17-M02). The EMA decision (P/0200/2020), including all annexes, issued on 25 May 2020 is also enclosed (001475-PIP03-17-M02 Decision with annexes).

The PDCO issued an opinion on compliance for the PIP: In accordance with Article 23(2)(a)(b)(c) of Regulation (EC) No 1901/2006, as amended, Mirum Pharmaceuticals requested the EMA to check as to whether Study 2/LUM001-501 has been conducted in compliance with the agreed PIP (EMEA-001475-PIP03-17-M02) as set in the EMA's decision P/0200/2020 of 25 May 2020. The EMA confirmed the PIP Study 2/LUM001-501 to be compliant as set out in the EMA's Decision (P/0200/2020) of 25 May 2020.

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

The finished product is presented as an oral solution containing 9.5 mg/ml of maralixibat as active substance.

Other ingredients are: Propylene glycol, Disodium ethylenediaminetetraacetic acid (EDTA) dihydrate, Sucralose, Grape Flavour and Purified water.

The product is available in 30 ml amber-coloured PET bottle with a preinstalled LDPE adapter and a HDPE child resistant cap with a foam liner. Three sizes of CE-marked dosing dispenser for oral use (0.5 ml, 1 ml and 3 ml) are provided with each bottle.

3.1.2. Active Substance

General Information

Maralixibat chloride ((4R,5R)-1-[[4-[[4-[3,3-Dibutyl-7-(dimethylamino)-2,3,4,5-tetrahydro-4-hydroxy-1, 1-dioxido-1-benzothiepin-5-yl] phenoxy] methyl]phenyl] methyl]-4-aza-1-azonia bicyclo[2.2.2]octane chloride) is a new active substance for which full documentation has been provided in module 3.2.S. and justification for a new active substance claim has been made (refer to separate assessment report). The API has chiral centres and exists in polymorphs. The stable form is consistently manufactured and is highly soluble in aqueous solution.

Manufacture, process controls and characterisation

The impurity profile of one of the starting materials is considered critical to downstream processing. The described synthesis affords starting material of high purity. Multiple manufacturers of one intermediate are foreseen. Developmental information (i.e. change of solvent) indicate that changes to the route of synthesis of the intermediate have significant impact on the API. Therefore, this starting material should be redefined to ensure that the decisive quality step is under GMP control to ensure life cycle management of the impurity profile (major objection). The other proposed starting materials have been adequately justified.

The information provided on the impurity profiles of synthesis intermediates is very detailed and indicates that the API manufacturer has solid knowledge and understanding of the synthesis route.

Design space studies have been initiated, however, no proven acceptable ranges (PARs) or normal operating ranges (NORs) have been established. A design space is currently not requested.

Full structural elucidation is provided for the API, maralixibat chloride. The impurity profile has been studied in detail throughout the manufacturing process including impurities originating from the starting materials and intermediates. More information on the risk for potential presence of nitrosamines is needed.

Specification, analytical procedures, reference standards, batch analysis, and container closure

Mostly, specifications are considered acceptable. Methods are adequately described. Method validation is very brief and supporting data is requested. In addition to primary stability batches, batch analysis data has been provided for several batches throughout development and details regarding any changes to test methods and specifications are described. Batch data provided for batches manufactured between 2015 and 2020 are comparable

Specifications comply mostly with ICH and EU requirements.

The quality of the container closure system is sufficiently documented and suitability is confirmed by stability studies in section 3.2.S.7.

Stability

Currently ICH requirements are not fulfilled for the 3 primary batches and the claimed re-test period is not justified. Supporting data, however, has been provided for three batches covering the re-test period under ICH conditions. These results and further results from supporting stability studies confirm that the API is stable and that both API sources are comparable.

3.1.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

The MRX oral solution, 9.5 mg/mL, is supplied in a 30 mL (1 ounce) amber colored PET (polyethylene terephthalate) bottle with a preinstalled low-density polyethylene (LDPE) adapter and a child resistant (CR) cap containing a foam liner. Three sizes of CE-marked dosing dispensers for oral use (0.5 mL, 1 mL, and 3 mL) are co-packaged in the secondary container closure system.

The history given shows that the drug substance has been developed for multiple indications including hypercholesterolemia and cholestatic diseases. Initially, capsules and tablets were developed until a

switch to a liquid formulation was made. Since pediatric patients (1 year of age and older) are the target patient population for the proposed cholestatic disease, an oral solution formulation was selected due to its flexibility for dose adjustment based on patient's body weight and the preference for this type of formulation in young children. As the active substance is highly soluble in water, the choice of an aqueous solution is acceptable. The choice of route of administration, dosage form, dosing needs/flexibility and excipients in the preparation and administration devices have been adequate discussed. The original formulation was compounded on a patient specific basis with a varying amount of maralixibat but fixed dosing volumes (FDV). The commercial drug product utilizes a formulation with fixed drug substance concentrations (FDSC) and variable administered volumes to achieve the desired weight-based dosing. These two formulations are identical in their qualitative composition, but differ in their quantitative composition. The following excipients are used: purified water, which serves as the solvent for the drug substance. Propylene glycol, which serves dual functions: co-solvent for the drug substance and preservative to inhibit the growth of microorganisms. Sucralose, which serves as the sweetener. Disodium ethylenediaminetetraacetic acid (EDTA) dihydrate, which is used as antioxidant to improve product stability by inhibiting the oxidative degradation of the drug substance. Grape flavor, which serves as taste masking agent. The applicant establishes the quality target product profile (QTPP) and identifies the critical product quality attributes (CQAs). However, this approach is only applied superficially and is not explored further. The manufacturing process is a simple process one that involves compounding, solution filling and sealing of the bottles. For scale up the manufacturing process was transferred. During pharmaceutical development two, slightly different, container closure systems were used during pharmaceutical development. The CCS-A (container closure system-A) is supplied with a separate bottle adapter which is installed by the caregiver/patient prior to its first use. The CCS-B contains a preinstalled bottle adapter that is inserted in the neck of the bottle during primary packaging. The CCS-B container is used for the commercial marketing of the product.

Manufacture of the product and process controls

The manufacturing process involves compounding, solution filling and sealing of the bottles.. The compounded solution is filled into 30 mL amber PET (polyethylene terephthalate) bottles, followed by installation of a bottle adapter and capped with a CR (child resistant) closure. The solution-filled bottle is labelled and placed inside a carton along with the package insert and dosing dispensers. Drug product is a multi-dose product, an aqueous solution with an active ingredient that is highly soluble in water. According to the corresponding guideline this is a standard manufacturing process. In general, manufacturing process is adequate described. More information should be given of possible intermediates (sub-batches, holding times), whether it is a continuous process and of the filling process (volume/filter area, flow rates or pressure and/or transmembrane pressure). Critical manufacturing steps and in-process controls were not really identified. More information needs to be provided. Concurrent validation will be performed for the Process performance qualification (PPQ) of drug substance. A traditional process validation is proposed. Section R.2 contains the prepared validation scheme including the sampling plan and acceptance criteria. Nonetheless, in section P.2 and section P.3 there are still some open concerns regarding an adequate manufacturing process and the control of the manufacturing process. Before a concurrent validation can be approved, these concerns should be adequately addressed so that it can be demonstrated that this manufacturing process effectively and reproducibly produces the drug product that meets the predetermined specifications and quality attributes.

Product specification, analytical procedures, batch analysis

In general, specifications are justified by batch analysis and stability results and comply with ICH and EU regulatory requirements. Limit of assay (limit should be tightened), of impurity (adequate qualification of this impurity has not been demonstrated in the drug substance part) and weight loss should be justified or adjusted accordingly. The parameter Disodium EDTA Dihydrate Assay has to be tested in any case for release as well as for shelf life. Adequate description of the analytical procedures has been provided. Analytical methods described are adequate to control the finished product on a routine basis. The identification, assay, and degradation products of MRX oral solution, is determined by a stability-indicating gradient HPLC method. Identification of maralixibat chloride is determine by using ultraviolet (UV) spectroscopy. The propylene glycol assay in the oral solution, is determined by HPLC with refractive index detection. The determination of disodium ethylenediaminetetraacetic acid (EDTA) dihydrate is performed by HPLC. Validation parameters provided in the summary reports are considered acceptable, however, raw data in order to judge whether validation has been performed correctly have not been provided. Validation reports of the quantitative methods should be supported with individual test results for linearity, precision, accuracy and quantitation. Representative chromatograms should also demonstrate sensitivity of the quantitative methods at LOD. Presented batch analysis data of primary stability, supportive stability, and relevant clinical batches of oral solution of multiple strengths demonstrate batch–to-batch consistency. Proposed container closure system demonstrates compliance with the relevant requirements and is adequate justified in section P.2. Proposed amber PET bottle provides adequate protection from light and microbial contamination. The containers proposed for routine storage (for commercial distribution, the CCS-B container is used) are those which have been used in the stability studies supporting the shelf life. The quality of the container closure system and the dosing dispensers is sufficiently documented and suitability is confirmed by stability studies in section 3.2.P.8. Data provided are adequate.

Stability of the product

Parameters tested for stability are description, maralixibat chloride assay, degradation products, propylene glycol assay, weight loss, and microbial quality. The test parameters differ from batch to batch. The storage conditions are defined by the applicant for the long term storage conditions with 2°C-8°C and 25°C/60%RH and for the accelerated storage conditions with 40°C/75%RH. This approach does not comply with the ICH Q1A Guidance and no justification has been provided as to why the product is tested for long term stability for storage in a refrigerator at 2°C-8°C. The stability data show that primary and supportive stability batches are stable at long term and accelerated storage conditions when packaged in the commercial container closure system. All stability results met the acceptance criteria applicable at the time of testing, no upward or downward trends were detectable. In-use stability study was carried out at 2°C-8°C. Data should be submitted that the solution is not stable after opening at temperatures of 25°C and that it is necessary to store the opened solution in the refrigerator. Freeze-Thaw study, forced degradation study and photostability study were performed according ICH guidelines.

The long term testing should cover a minimum of 12 months' duration on at least three primary batches at the time of submission and should be continued for a period of time sufficient to cover the proposed shelf life. Currently ICH requirements are not fulfilled.

Since the stability studies have been performed using the conventional ICH storage conditions and the drug product is packaged in a semi-permeable container, the potential of water loss should be evaluated under conditions of low relative humidity as stipulated in guideline ICH Q1A (major objection).

3.2. Non clinical aspects

3.2.1. Pharmacology

Maralixibat chloride is a potent inhibitor of the apical sodium-dependent bile acid transporter (ASBT), localized on the luminal surface of ileal enterocytes, which mediates uptake of conjugated bile acids (BAs) across the brush border membrane of the enterocyte.

In vitro pharmacology

In vitro in maralixibat inhibited [^{14}C]taurocholate uptake using an incubation period of 2 hours with an IC_{50} value of 0.28 nM. The in vitro proof of concept is considered established, however, binding characteristics including potency of maralixibat and sequence homology of ASBT across non-clinical animal species used in in-vivo PD and toxicology studies was not presented. This should be clarified (**OC**). Compared to alanine transport, for maralixibat a selectivity for BA transport in was 127,500:1. The Applicant is asked to scientifically justify the choice of the alanine uptake as a base for the calculation of a selectivity factor of maralixibat for the uptake of taurocholate (**OC**). The interaction of maralixibat with taurocholate was shown to be competitive and the inhibition of the ASBT was demonstrated to be reversible while exhibiting some time dependence, i.e. the potency of inhibition increased over the first ten minutes of incubation. In expressing human ASBT an IC_{50} value of 7.04 nM was determined for maralixibat, the short incubation time of 5 min used in this experiment considered to be likely the cause for the higher IC_{50} value determined.

In vivo pharmacology

The pharmacodynamic effects of maralixibat in vivo were investigated in naïve rats, partial bile duct ligated (pBDL) rats as a cholestasis model, dogs and monkeys. While rats lack gall bladders, dogs and monkeys do like humans possess gall bladders.

Naïve rat

In vivo in naïve male Wistar rats, oral treatment with maralixibat of up to once daily 2 mg/kg for 4 days resulted in a dose-dependent increase in fecal bile acid (fBA) excretion up to 3.6-fold with an ED_{50} of approximately 0.027 mg/kg/day. Thus indicating that maralixibat is able to block ASBT-mediated BA transport in the rat intestine leading to increased fecal BA excretion. In naïve Sprague-Dawley rats administered orally once daily up to 150 mg/kg/day of maralixibat for up to 4 weeks (as part of a toxicology study), an increase in total fBA levels at Week 2 an up to 11.1-fold (sex-combined) over control at 150 mg/kg/day dose was seen.

Rat model of disease

The ability of maralixibat to reduce serum bile acids (SBA) and prevent liver injury was investigated in the rat partial bile duct ligation (pBDL) model of cholestasis. Maralixibat is intended to treat patients suffering from PFIC2, which is caused by a malfunction/loss of function of the BSEP. The Applicant is asked to comment on the appropriateness of the pBDL rat as the model chosen for in-vivo PD (**OC**). In pBDL male Sprague Dawley rats treated orally once daily with up to 10 mg/kg maralixibat statistically significant reductions in sBA and ALT were seen at Day 3, the first time of sampling, and reductions in additional liver injury biomarkers at 7 and 14 days. Fecal BA excretion was increased already at the low dose of 0.3 mg/kg/day up to 6-fold at Day 10.

The same pBDL rat model was utilized to evaluate the effect of maralixibat in combination with UDCA on BA reabsorption. Male Sprague-Dawley rats were dosed once daily by oral gavage with vehicle, maralixibat alone (1 mg/kg), UDCA alone (1 mg/kg), or maralixibat in combination with UDCA (1 mg/kg for both). Ursodeoxycholic acid helps regulate cholesterol by reducing the rate at which the intestine absorbs cholesterol molecules while breaking up micelles containing cholesterol. Since UDCA presents with a different mechanism of action, it seems prudent to evaluate co-administration in an animal model. 7-14 days after ligation, beneficial effects were shown on excreted fBA and serum biomarkers for liver disease for both maralixibat, UDCA and the combination of the two compounds indicating that the effect could be additive. No justification for choice of dose of UDCA (1 mg/kg/day) was presented. The human dose is 12-16 mg/kg/day (UDCA SPC). This should be clarified (**OC**).

When maralixibat was compared to obeticholic acid (OCA) 1 or 10 mg/kg maralixibat increased fBA excretion statistically significant when investigated at Day 10 while a slighter increase in fBA excretion after treatment with up to 30 mg/kg OCA did not reach statistical significance. Likewise, already 1 mg/kg/day maralixibat reduced at Day 14 of treatment sBA, AST, ALP, GGT and total bilirubin statistically significantly in serum, while only 30 mg/kg/day of OCA reduced statistically significantly serum AST and ALP. The dose of 10 mg/kg/day of maralixibat did show for all in serum investigated parameters less pronounced effects than the ten times lower dose of 1 mg/kg/day. The Applicant is asked to comment and to relate the in vitro determined IC_{50} values for maralixibat with the estimated ileal maralixibat concentration in the rat (**OC**).

In vivo Pharmacology in dogs

In Beagle dogs after oral daily treatment with 1 or 4 mg/kg of maralixibat for 14 days statistically significantly dose-dependent increases in fBA excretion (up to 5-6 fold compared to pretreatment) and statistically significant reductions in serum total and HDL-cholesterol were seen. The Applicant is asked to comment whether reduction of serum cholesterol is a pharmacologically desired effect in the population intended to be treated (**OC**).

The increase in fecal BA excretion was similar when maralixibat was administered 30 minutes prior to or 4 hours after feeding. A single dose of maralixibat 30 minutes before a meal produced a dose-dependent inhibition of the postprandial rise in serum bile acids (sBA) with the maximum inhibitory dose being approximately 2 mg/kg (about 90% inhibition) and the interpolated ED_{50} being 0.2 mg/kg. For comparison, the human dose is 266 µg/kg QD (SmPC), HED for ED50 in dog = 0.54 *0.2 mg/kg = 0.108 mg/kg. Hence, the human dose is well above ED50, if the potency of maralixibat is comparable between dog and human ASBT.

When the onset of maralixibat induced inhibition of the postprandial rise in sBA was investigated maralixibat in single oral doses of 0.05, 0.2 and 1 mg/kg, given 1 hour after feeding, statistically significantly lowered sBA levels starting 1 hour after administration of 0.05 mg/kg and 30 minutes after administration of the two higher doses. When the duration of the reduction of the postprandial sBA rise was investigated, it could be demonstrated that a single dose of 0.2 mg/kg resulted in a longer reduction in sBA levels than the single dose of 0.05 mg/kg did.

In vivo pharmacology in monkeys

In cynomolgus monkeys administered oral doses of maralixibat once daily for 7 days, fBA levels were increased by 2- and 5-fold at doses of 5.0 and 20.0 mg/kg, respectively. There was no effect on fBA excretion at doses of 1.0 and 2.5 mg/kg. The Applicant is asked to comment what is known about species differences in the magnitude of PD effects of maralixibat in the gallbladder positive species dogs and monkeys and in humans and to assess what the most relevant species in toxicology studies is (**OC**).

Overall, in vivo proof of concept is considered well established in both rat, dog and monkey. However, the monkey appeared less sensitive to the effect of maralixibat.

Secondary pharmacodynamics

No studies investigating secondary pharmacology have been submitted. The absence of secondary PD studies on targets outside the gastrointestinal system is considered acceptable, since the systemic exposure to maralixibat is negligible. However, the concentration in the gut, especially in the stomach, may be high and other transporters/enzymes/receptors than ASBT in the gut may be affected. In addition, data provided (alanine transporter only) is not considered sufficient for claiming high specificity of maralixibat for the ASBT. Applicant is asked to provide scientific argumentation on this concern and conducting a screen on receptors and other appropriate molecular structures in the gastrointestinal system including the stomach is recommended (**OC**).

Safety Pharmacology

In vitro

According to guideline ICH 7A the safety pharmacology core battery should ordinarily be conducted in compliance with GLP. The Applicant is asked to justify why the in-vivo safety pharmacology studies were not performed compliant to GLP regulations (**OC**).

The Applicant submitted a study report on an in vitro hERG channel assay. Due to several mishaps occurring during the conduct of the study the data obtained are considered limited. Although the hERG study was claimed to GLP compliant, presence of maralixibat in test article formulations, could not be verified, even though the concentration was intended to be in the range of 200-700 ng/mL (0.3 – 1 µM). Since maralixibat is fully soluble in water. The Applicant is asked to discuss and justify the validity of the submitted study (**OC**).

In vivo Cardiovascular Safety

Cardiovascular safety studies were conducted in dog after both oral (conscious) and intravenous administration (anaesthetised), however, no study was GLP compliant. Radiotelemetry implants were previously implanted for measuring hemodynamics and electrocardiograms in dogs dosed with oral capsule. Both studies were supported with exposure determination (plasma concentration) and may be considered valid. After oral dosing no cardiovascular changes were observed on CV hemodynamic or electrocardiograph parameters for doses up to 20 mg/kg. HED of 20 mg/kg = 20 *0.54 = 10.8 mg/kg providing a safety margin of 10.8 mg/kg: 0.532 mg/kg = 20.3. After intravenous administration providing plasma concentrations in the µg/mL range, no clinically relevant changes in electrocardiographic parameters were observed. Hence, maralixibat is not expected to impact cardiovascular safety.

CNS Safety

The CNS safety study is not GLP compliant. Nevertheless, the study design and interpretation of data appear to be acceptable. Maralixibat administered to rats at once daily oral doses of 150 mg/kg or once daily IV doses up to 0.3 mg/kg for 4 days was tolerated except for salivation observed in animals dosed orally at 150 mg/kg. No maralixibat-related effects were noted for the functional observation battery (FOB; home cage and open field activity and tests of reflexive, physiologic, and neuromuscular

function) or locomotor activity. The salivation is not considered relevant, since the dose of 150 mg/kg is much higher than clinically relevant (HED = 150 * 0.16 = 24 mg/kg). The IV doses were chosen to be 2-3 orders of magnitude higher than human systemic exposure after an oral dose of 1 mg/kg. Testing was performed immediately after the intravenous bolus dose. Therefore, the safety margin to systemic exposure can be considered sufficient. A PK study in rats evaluating an intravenous dose of 3 mg/kg is considered supportive of exposure in the CNS safety study.

It should be noted that although at the very high dose of 5 mg/kg of maralixibat IV administered to dogs in the course of an acute toxicity study signs of slight lethargy, tremors and muscle stiffness had been reported. These effects are not considered clinically relevant due to extremely large differences in exposure between the IV treated dogs in that experiment and therapeutically orally treated humans.

Respiratory Safety

Anesthetized male guinea pigs were subjected to single chamber plethysmographs to evaluate potential effects of maralixibat on airway resistance and dynamic lung compliance. The animals were dosed by IV bolus with 0.39 mg/kg and a maintenance dose of 1.9 mg/kg over 45 minutes. No significant effects were observed when compared to placebo.

Pharmacodynamic Drug Interactions

Co-medication with UDCA was evaluated in a rat study. It appears that co-administration with UDCA could be beneficial.

Co-administration of maralixibat with other medication used in the clinical setting was not discussed. These include oral bile acid binding resin, cholestyramine and rifampicin. Options to relieve pruritus include antihistamines, steroids and naltrexone (Clinical Overview). A discussion on potential implications of co-administering maralixibat with bile acid binding resin, cholestyramine and rifampicin should be presented **(OC)**.

3.2.2. Pharmacokinetics

Methods of analysis

Maralixibat has been in development for more than two decades and a long list of general toxicity studies has been submitted. To limit the number of studies for bioanalytical assessment, a list of studies considered the most important is provided (please refer to Table 3.1.2 in CoRapp´s AR) with comments on bioanalytical method validation status (please refer to Table 3.1.3 in CoRapp´s AR).

The Applicant (Mirum) sponsored new general toxicity studies in rodents. All dog studies were conducted. Embryofetal development was evaluated in rabbit . For these two studies, no separate bioanalytical report was provided, however that as well as ISR was not required at the time of study conduct. Moreover, both studies include a QA statement documenting audit of bioanalytical raw data and toxicokinetics draft reports.

The bioanalytical program appears to be and have been in good control with robust bioanalytical methods and documented stability of maralixibat in plasma. From the final reports, some documents appear to be missing. Therefore, for completeness of the bioanalytical documentation, the validation report and amendment (KV95CJ and 12-8930(a)) supporting the most recent studies in adult and

juvenile rat (MRX NC-001 and MRX-NC-004) should be submitted for assessment. Moreover, documentation of ISR in adult rat should be provided as well (OC).

Validation studies were provided for determination of maralixibat in plasma of TgrasH2 and CD-1 mice, rats (juvenile and adult), rabbits and dogs. Plasma concentrations of maralixibat were measured. Plasma concentrations of radiolabeled maralixibat were analyzed was also developed and validated for quantifying maralixibat concentrations in diet admix formulations and water.

The analytical results of some studies (SA4892; SA4896, A9102M-SHP625) indicated that "SD-5613 sticks to the glassware and dosing apparatus. Therefore, an additional correction factor was applied." In this case an acute toxicity study is concerned, which would not require a separate validation study. However, the justification of this "additional correction factor" remains questionable and the applicant is requested to provide an overview of the studies where a correction factor was applied and to provide a sound justification for the factor used. Furthermore, the applicant is asked to comment on the influence of the adherence of maralixibat to surface areas and the results obtained on maralixibat concentrations in the different studies (**OC**).

Pharmacokinetics of maralixibat was investigated in numerous studies in mice, adult and juvenile rats, pregnant and non-pregnant rats, pregnant rabbits, guinea pigs, dogs and monkeys. The applicant limits the presentation of results on absorption in the Pharmacokinetics written summary (Module 2.6.4) to the studies/ study parts with oral administration. Results on studies/study parts with intravenous administration, although conducted, are not provided. Some results of studies /study parts obtained after intravenous administration can be found under the subsection 2.6.5.16 Pharmacokinetics: Other (IV.single dose), whereas the respective section of the Pharmacokinetics written summaries (2.6.4.8 Other Pharmacokinetic Studies) indicates, "No other PK studies were conducted". The summary of data concerning this aspect has to be considered to be inconsistent and incomplete and the applicant is therefore requested to update the dossier accordingly *(***OC***).*

Concerning study 4988 the applicant presents data which do not completely comply with the study report. The applicant is therefore requested to justify the given tmax data (OC).

Another issue concerns the presentation and discussion of results on the volume of distribution, which is limited to results after intravenous administration although data for oral administration is available, which show a much higher volume of distribution. Further, there are indications of a dose dependency; the applicant is, therefore, requested to present its conclusions regarding this issue (**OC**). In two studies in juvenile animals, (Studies MRX-NC-001 and 13-4397) maralixibat was found in control or pre-dose animals. A detailed error discussion is currently missing and the applicant´s approach does not concur with the Guideline on the evaluation of control samples in nonclinical safety studies: Checking for contamination with the test substance" (**OC**).

In addition, several minor inaccuracies were noticed, which are discussed in the respective section of the Day 80 AR. Further actions are not required in these cases.

Absorption

Absorption after single or repeated dose oral administration was investigated mice, juvenile and adult rats, (pregnant) rabbits and dogs in a multitude of studies.

Absorption after single dose

Single dose bioavailability was evaluated in mouse (50, 150 mg/kg), rat (1- 2000 mg/kg), rabbit (50, 150 mg/kg) and dog (1-1000). Bioavailability was below 1% in all species and tested doses. A study in

femoral artery and portal vein cannulated rats showed that first pass effect was not the cause of the low bioavailability as it only accounted for 7.88% of the administered dose as determined from AUC after intravenous and intraportal infusion. A similar study design was used in dog, where first pass effect and/or biliary excretion actually was shown to affect the bioavailability by up to 79% (M3098258 and M3098260).

Absorption in dog was evaluated both after oral gavage and oral capsule. The capsule formulation increased T_{max}, however bioavailability was still below 1%. In an acute toxicity study, dogs were dosed 50, 200, 400, 600, 800 or 1,000 mg/kg maralixibat as an oral capsule (SA4948). Emesis increased with dose and bioavailability decreased with dose (range 0.08 to 0.02%).

Absorption after repeat dose

An extensive amount of repeat-dose pharmacokinetic studies was conducted to evaluate the absorption of maralixibat in all relevant species including pregnant rabbits and juvenile rats. Low bioavailability was observed in all species except the youngest juvenile rats. Low bioavailability is also evident in humans including children.

Repeat dose in mouse

Repeat-dose TK of maralixibat in mice was evaluated in 9 studies of 2 to 26 weeks duration. 5 studies were using oral gavage and 4 were using dietary admix. The dose-range for oral gavage was 2.5 to 2.000 mg/kg/day and for dietary admix 5 to 10.000 mg/kg/day. Bioavailability was less than 1% for oral gavage and generally even lower for dietary admix. Females showed higher exposure than males in the final 26 weeks carcinogenicity study in rasH2 mice at doses 7.5 and 25 mg/kg/day on Day 1 (MRX-NC-002). Other studies show the same tendency at doses up to 250 mg/kg/day (M8562M-SHP625 and M7614M-SHP625). High accumulation was evident in some dosing groups in study MRX-NC-002.

Repeat dose in rat

Repeat-dose TK in rat was evaluated in 10 studies of 2 to 13 weeks duration in adult rats. 8 studies were using oral gavage and 2 were using dietary admix. The dose range for oral gavage was 1-1.500 mg/kg/day and for dietary admix 150 to 2.000 mg/kg/day. Bioavailability was less than 1% for oral gavage and generally even lower for dietary admix, typically less than 0.1%. No obvious sex differences were observed and slight accumulation was only seen at very high doses in female animals.

In the recent 13-week study sponsored by Mirum (MRX-NC-004), the following was concluded:

No appreciable sex differences observed. The values for C_{max} were 1.06, 9.76 and 43.1 ng/mL on Day 1 and 1.16, 17.4 and 48.9 ng/mL on Day 91 for the 10, 300 and 1,000 mg/kg/day doses, respectively. The values for $AUC_{0\text{-}24hr}$ were 8.70, 129 and 609 ng·hr/mL on Day 1 and 12.2, 129 and 627 ng·hr/mL on Day 91 for the 10, 300 and 1,000 mg/kg/day doses, respectively.

As dose increased, C_{max} and $AUC_{0\text{-}24hr}$ also increased, though at a less than dose proportional manner from the 10 to 300 mg/kg/day oral dose. The increase was dose proportional from the 300 to 1,000 mg/kg/day dose. The accumulation ratio was 1.40, 1.38 and 1.03 for the 10, 300 and 1,000 mg/kg/day doses, respectively, which suggests that accumulation is low following repeated doses.

Toxicokinetics was evaluated in juvenile rat from PND7 to PND 21 (MRX-NC-001) and from PND21 to PND63 (13-4397). The highest exposures were documented in the youngest rat pups on PND 7, and

decreased significantly on PND 14 and 20. Bioavailability was approximately 17% on PND 7, but decreased to <1% by PND 20. This decrease in bioavailability over the course of the experiment was likely due to decreasing permeability of the maturing GI tract in the rat pups during the early neonatal period. A concern regarding the clinical relevance of the high bioavailability in the youngest rats is raised in the clinical section.

In the older juvenile rats, C_{max} and $AUC_{0\text{-}24hr}$ values were similar or greater on PND 56 compared to PND 21. There was some accumulation observed in female rats (accumulation ratio between 1.6 and 1.8) after repeated doses, which may be in part due to the higher doses administered compared to the doses administered to male rats (25-, 50-, 100-, or 200-mg/kg/day dose to males and females were administered either a 125-, 250-, 500-, or 1,000-mg/kg/day dose).

Toxicokinetics after dietary admix was evaluated in pregnant rats in two studies; SA5043 (50, 250 and 1000 mg/kg/day) and EX5034 (250, 750 and 2,000 mg/kg/day). Exposure was documented; however, bioavailability was in the range of 0.04 to 0.25%.

Repeat dose in rabbit

Repeat-dose TK in pregnant rabbit was evaluated in two studies (EX5033 and SA5061) covering the dose range of 25 to 500 mg/kg/day. In both studies rabbits were dosed from GD7 to GD18 and the bioavailability of maralixibat was < 0.1% across all dose ranges and days of sampling. Exposure was too low on GD7 for the lower doses to conclude anything on dose proportionality, however exposure was much higher on GD18 than on GD7 for the doses 100 and 250 mg/kg/day. Exposure was higher than dose proportional on GD18 in study SA5061 reaching 766 ng/mL*h for the highest dose of 250 mg/kg/day. It should be noted that rabbits receiving 500 mg/kg/day in the DRF study (EX5033) did not survive to GD18.

Repeat dose in dog

Repeat-dose TK in dog was evaluated in studies of up to 1-year duration. In general, bioavailability was less than 0.2% across the whole dose range of 1 to 600 mg/kg/day. Dogs suffered from emesis, hence toxicokinetics may be less reliable, as the fact that many samples were below LLOQ, also show. The highest $AUC_{0\text{-}24h}$ and C_{max} was reached on Day 176 at 100 mg/kg/day in the 1-year toxicity study (455 ng/mL*h and 113 ng/mL, respectively). Similar range of exposure was obtained in the 13-week toxicity study at the same dose level (SA4991). Accumulation was observed at higher doses (100 and 300). No sex differences in exposure were observed in any study. Exposure increased with increasing dose, however dose proportionality evaluation is deemed unreliable due to the large variability and many samples being >LLOQ. In the 1-year study doses up to 20 mg/kg/day was relatively well tolerated, providing $AUC_{0\text{-}24h}$ and C_{max} of 57.7 ng/mL*h and 18.5 ng/mL, respectively (SA4987). Plasma concentrations in patients are often below LLOQ of 0.25 ng/mL (SmPC).

Distribution

Distribution was investigated in (pigmented) rats and dogs by the use of [^{3}H] and [^{14}C] labelled maralixibat.

Tissue to plasma ratios of tissues in the gastro intestinal tract was in the range of 100 to 6000 in the rat after 5 mg/kg of ^{14}C-maralixibat (M2098359). Levels of ng equivalents/g in tissues of the gastrointestinal tract was largely in a similar range between rat and dog after oral doses of 5 mg or 7.5 mg/kg of ^{3}H-maralixibat, however levels in female rats was lower than in male.

Due to maralixibat's low bioavailability, distribution to other tissues is low, although maralixibat was also found in the liver and pancreas of the rat at levels approximately 10 times higher than plasma. Maralixibat was detected in many other tissues in low amounts, but at the last time point of 168 hours, the highest concentration was still in the small intestine, hence no retention in specific organs was observed. No binding to skin was detected (non- or pigmented skin).

Other tissues than gastrointestinal were not evaluated in the dog.

Plasma protein binding was determined with [^{14}C]maralixibat for mouse, rat, guinea pig, rabbit, dog monkey and human (M3099225), covering the concentration range of 0.25 to 25.0. µg/mL. Plasma protein binding was high (99 - 84%) and concentration independent in all species tested. Partitioning of 0.250, 2.50 and 25 µg/mL maralixibat into red blood cells of rats (20 – 43%), dogs (22 – 38%) and humans (33 – 45%) was determined by the use of [^{14}C]maralixibat in-vitro. Partitioning into blood cells appears to be dose dependent over the concentration range tested. Results of in-vivo studies showed, that maralixibat does not preferentially distribute into red blood cells (in vitro:–M3099280; in vivo: M3099009, M3098328 and M3098330).

Regarding the volume of distribution the applicant refers to studies with IV administration performed in mice, rats, guinea pigs, dogs and monkeys. However, data on distribution after oral administration is available (please, refer to the OC above). In mice and dogs, the apparent volume of distribution at steady-state (V_{ss},0.622 and 0.257 L/kg, respectively) after intravenous application was lower than total body water/ total extracellular fluid indicating that maralixibat did not penetrate extensively into tissues. For rats and monkeys, the apparent V_{ss} (0.73 and 0.599/0.296 ml/kg. resp.) and the volume of distribution (Vz) following intravenous application were slightly higher than the volume of total extracellular water. Furthermore, the applicant claims that the volume of distribution and the volume of distribution at steady state for guinea pigs were slightly higher than the volume of the total extracellular/ body water without providing a reference. Since studies in guinea pigs are limited to studies on excretion and local tolerance, this missing information is considered to be of low relevance and does not justify a point of concern. Of rather more importance is, that the applicant limits the overview on studies with intravenous administration for unknown reasons, although data on oral administration is available (point was already raised above). In at least 2 studies in mice (M8562M-SHP625; M7614M-SHP625) the volume of distribution was calculated after oral administration. In both studies, a much higher volume of distribution was found compared to intravenous administration. Furthermore, in study M8562M-SHP625 the volume of distribution appears to be dose dependent. The applicant is asked to present its conclusions regarding this issue (**OC**). No information is provided regarding a potential transfer of maralixibat into the milk or the developing fetus. This appears to be acceptable in the light of the low bioavailability and the resulting low plasmatic levels anticipated in human use.

Metabolism

In vitro

Metabolism was investigated in vitro and in vivo. In-vitro metabolism studies were performed in liver microsomes of rat, dog, cynomolgus monkeys and human using [^{14}C]maralixibat. Several metabolites have been identified. Studies with subsequent HPLRC identified potential sex differences in rats only. 4 potential metabolites were detected in males and 3 in females. Metabolism rate was low (12.3 % in males and 2.4 % in females). In dogs, at least 7 potential metabolites were identified. The metabolism rate was slightly higher (21%) compared to rats. Microsomes obtained from Cynomolgus monkeys showed the highest similarities to metabolism in humans. Metabolism rate was higher (74% and 69% in Cynomolgus monkeys and humans, respectively) compared to rats and dog. In both species, at least

8 potential metabolites were identified. By subsequent LC/MM/MS and LC-MR detection, 6 potential metabolites (M1-M6) were detected. These metabolites included the N-demethylation metabolites of maralixibat (M1 and M2) and the hydroxylation metabolites of maralixibat (M3 and M6), as well as the N-demethylation and hydroxylation metabolites M4 and M5. Based on this data the applicant has proposed an overview on potential metabolic pathways in liver microsomes. In the light of the low bioavailability and the rather local mode of action the relevance of the discrepancies between human and the species involved in toxicity testing is of minor relevance.

In vivo

A potential pre-systemic metabolism was investigated in mice, rats, rabbits and (female) dogs. Maralixibat was mainly detected in the faeces. In mice one minor degradation product was identified in the faeces (N-demetylated maralixibat; M1) and in rabbits three (M1 (N-demethylated maralixibat); M2 (N-di-demethylated maralixibat) and M3 (monohydroxylated maralixibat)). In rats and dogs, maralixibat appears to be stable. Systemic metabolism was investigated in rats and dogs following intravenous administration. In rats, sex-related difference in the metabolism were noted. In female rats, only 2.07% of the dose was metabolised compared to 55.0% in male rats. In dogs the majority of the dose was excreted in the feces as parent compound (65.1%) with a smaller amount excreted as metabolite M3 and one other minor degradation product/metabolite M1 (total metabolites = 8.95%). No apparent sex-related difference in metabolism was noted. Keeping in mind maralixibat´s low bioavailability in all species including humans, potential differences in the metabolic patterns of the species involved in toxicity testing has to be considered of minor relevance. The omission of studies of Phase II metabolism and induction/inhibition of CYPs is considered acceptable. From pharmacokinetic point of view toxicity testing in mice, rats, dogs and rabbits is therefore adequate

Excretion

Excretion of maralixibat following single dose administration was investigated in mice (oral), rats (oral and intravenous), guinea pigs (intravenous), rabbits (intravenous), and dogs (oral and intravenous) (M3000146, M2000269, M2000112, M3099010, M2000113, M2098132 and NB4-02-06-004). Excretion after oral repeated dose administration was investigated in mice, rats and dogs (M3000146, M2000269, M2000114). Maralixibat was mainly excreted via feces (71.9 – 96.4%) . Only very low amounts could be detected in urine (0.1 to 9.63%). Adequate recovery was obtained in all these studies for the conclusions to be considered valid (72.5 to 96.6%).

Comparisons with published data (Davies and Morries 1993) showed that the clearance was lower than the hepatic blood flow in mice, rats, dogs and monkeys. It can, therefore, be concluded that, following intravenous administration the excretion of maralixibat is not limited by the hepatic blood flow. The applicant claims the same for the guinea pig without providing published evidence. Since the relevance is rather low, further actions are not required.

No robust pharmacokinetic drug interaction studies were submitted within the non-clinical part of the dossier. The Applicant claims completeness of the investigations concerning this point and refers to the clinical part of the dossier. There are no objections against the Applicant´s approach to place this aspect in the clinical part (For further information on this aspect, please, refer to the clinical part of this report).

3.2.3. Toxicology

The non-clinical toxicology programme submitted by the Applicant is considered to be in general in line with the respective guidelines.
Module 2.6.7 "Toxicology Tabulated Summary" was found to contain rather many mistakes throughout the complete module. As the assessment of the data is based on the documentation provided by the Applicant an **OC** is raised regarding this module.

The batches of maralixibat used for the toxicology program were synthesized using the same synthetic route as that used for clinical study batches and that planned for commercial batches, with impurity profiles representative of the clinical API.

The nonclinical PK data indicate that maralixibat is minimally absorbed after oral administration. Since systemic exposure following oral administration of maralixibat is low, findings from IV administration are not likely to be clinically relevant.

Toxicology studies with maralixibat (salt form) were conducted in mice, rats, dogs, rabbits, and monkeys. For the main nonclinical toxicity assessment, Sprague-Dawley rats and beagle dogs were used, and those were also the strains used for key ADME studies in rats and dogs. Various formulations of maralixibat API were used, i.e. a simple aqueous solution of maralixibat API in water for oral gavage of small animals, dietary admix formulations were used for some repeat-dose toxicity studies and maralixibat API in capsules was utilized for dosing of dogs.

Single dose toxicity

Single dose toxicity studies were performed in the rat and in the dog with oral and with IV administration of maralixibat.
In the oral study in rats doses of 0, 1000, and 2000 mg/kg of maralixibat were administered via gavage. The minor maralixibat-related adverse effects observed, which were reversible within one week, included stool alterations and salivation.
In the rat study using the IV route doses of 0, 0.06, 0.6 and 6.0 mg/kg were administered. Transient clinical signs beginning 10-15 min post-dose were observed at the high dose only, were transient and included ataxia, reduced activity, reduced body tone, tremors and dilated pupils.

In the GLP compliant oral single dose toxicity study in Beagle dogs doses of 0, 50, 200, 400, 600, 800, or 1,000 mg/kg were administered. Emesis was observed at doses of ≥ 50 mg/kg and was considered severe at doses of ≥ 400 mg/kg. Stool abnormalities were observed at doses of ≥ 200 mg/kg. Exposure values were rather similar in the 400 to 1000 mg dose groups, possibly due to either emesis or saturation of absorption.
In the single dose toxicity study in Beagle dogs with IV administration doses of 0, 1, 2.5 and 5 mg/kg of maralixibat, transient clinical neurological signs of lethargy, tremors or muscle stiffness were noted at the highest dose. As the exposure (C_{max}) is more than 70,000 times higher than the C_{max} reported for humans receiving more than 2 times the clinically intended maximum therapeutic dose for two weeks, the neurological signs seen in the dog are considered of no clinical relevance.

Repeat-dose toxicity

Repeat-dose toxicity studies were performed in mice for up to 13 weeks, in rats for up to 26 weeks, in dogs for up to one year and in the monkey for 2 weeks. The durations of the chronic repeated dose toxicity studies is in line with ICH M3. The route of administration was the oral route, the route of administration in humans. A total of 27 repeated dose studies were performed. A part of them aimed

at finding an appropriate dose for carcinogenicity studies in mice. In mice and rats, a part of the studies was conducted using gavage and another part was conducted using dietary admixture. Bioavailability of maralixibat in the repeated dose studies was generally below 1%.

The three GLP compliant repeated dose toxicity studies in CD-1 mice were conducted with treatment durations of 13 weeks. In two of these studies maralixibat was administered via oral gavage, one via admixture to the diet. Dosages in the studies using gavage were 0, 50, 150, 500, 1000 mg/kg/day and 0, 50 250, 750 mg/kg/day, respectively, in the study using dietary admixture 0, 50, 150 and 750 mg/kg/day were administered. In the studies using gavage deaths were mostly attributed to gavage errors/aspiration or respiratory distress due gaseous distended abdomen especially in high dose groups. The latter effect is considered to be possibly related to a change in the intestinal microenvironment due to administration of maralixibat. Reduction in bodyweight/- gains have been seen predominantly in male animals and at higher dosages. A consistent finding in the studies is prolongation of coagulation parameters (PT, aPTT) in males starting from the lowest dose of 50 mg/kg/day of maralixibat (gavage or admix). The Applicant considers this effect likely to be caused by vitamin K deficiency. In the mouse repeated dose toxicity studies no bleeding events were reported. Increases in cecum and/or colon weights are reported in both genders in all studies and they are possibly related to the oral administration of poorly absorbable material. Increases in fecal bile acid excretion, the expected main pharmacological effect of maralixibat, were seen in all of these studies. In the studies using gavage, increases in serum cholesterin/HDL are reported at the highest administered doses. These effects, although not expected as a direct pharmacological effect of maralixibat, according to the Applicant increases of serum cholesterol in rodents treated with certain cholesterol-lowering drugs (like the statins) have previously been described and are thought to be related to strong induction of HMG-CoA reductase.
The NOAEL in the mouse gavage study SA4954, the study of the two gavage studies with more parameters investigated, is considered to be the MD of 150 mg/kg/day. Mean combined C_{max} was 71.7 ng/mL and $AUC_{0\text{-}24hr}$ was 529 hr*ng/mL. In humans after administration of once daily doses of 100 mg maralixibat for 14 days, mean C_{max} was 1.146 ng/mL and mean $AUC_{0\text{-}24}$ was 4.614 ng•h/mL were reported. As this dosage in humans is about twice the clinically maximally intended one, assuming dose linearity, exposure based safety factors between NOAEL in this study and the maximum clinical dose of at least 91 (C_{max}, male animals) and 207 (AUC female animals) result. The NOAEL in the mouse study SA5005 with admixture to diet is considered to equal the HD of 750 mg/kg/day. A mean gender-combined C_{max} of 21.9 ng/mL and $AUC_{0\text{-}24hr}$ of 453 ng•h/mL were reported which would under the same assumption result in exposure based safety factors of at least 37 (outlier-eliminated C_{max} in male animals) and 186 (outlier eliminated $AUC_{0\text{-}24hr}$ in male animals) result. Considering the toxicological effects reported in the repeated dose toxicity studies with maralixibat in mice these exposure based safety factors are considered sufficient.

Six GLP-compliant repeated dose toxicity studies in rats were performed with a treatment duration of 13 weeks/3 months. In five of the 13 week studies maralixibat was administered orally via gavage, in the remaining one and in the 26 week GLP compliant study via dietary admix. Three of the gavage studies and one of the dietary admix 13 week studies included recovery periods of 4 weeks. The maximum dosages in the gavage studies ranged from 150 mg/kg/day to 1500 mg/kg/day. In two of these studies males received up to two thirds lower dosages than females. In the dietary admix study of 13 weeks duration the highest dose level was 1500 mg/kg/day. In the 26 week dietary admix study the dose levels were initially 0 (males + females), 30 (males only), 150 (males + females), 500 (females only), 750 (males only), and 2000 (females only) mg/kg/day of maralixibat. Due to high mortalities in HD groups, the dose in HD males was lowered around week 12 from 750 to 300 mg/kg/day and in the group of HD females around week 12 from 2000 to 1500 mg/kg/day of maralixibat.
In general, in the studies with administration of maralixibat via oral gavage a considerable number of

deaths were attributed to aspiration of dosing solution. In all but two repeated dose toxicity studies in rats, especially in male animals, prolongation of coagulation parameters with bleeding events at higher dosages were seen. An **OC** is raised regarding the possible cause for the obvious lack of such reported findings in studies R7834 and MRX-NC-004. The lowest dose at which prolongation of PT and aPTT was already seen, was 5 mg/kg/day of maralixibat (study SA4865) and at 500 mg/kg/day (study SA5006) fatal bleeding events occurred in male animals. When investigated, the prolonged coagulation times showed reversibility. The Applicant considers secondary to loss of bile acids induced vitamin K deficiency a likely cause for the prolongation of coagulation times/bleeding events. Prolongation in coagulation time was only considered an adverse effect by the Applicant, if it was associated with bleeding event. This is not endorsed, as increasing PT and APTT precedes the bleeding event and the increasing values should therefore be considered an adverse effect in itself. However, as vitamin K deficiency and prolonged coagulation time appears to be rodent specific and not a problem at clinically relevant doses in dog, monkey or human, the issue will not be further pursued. Nevertheless, it should be sufficiently described in the SmPC (see **OC** below).

Another phenomenon/clinical observation associated with maralixibat treatment is the discolouration of papers placed under the cages or unusual urine colour. In one study this was already seen at a dose as low as 5 mg/kg/day and studies hint that this phenomenon is more prominent in MD than in HD animals. The Applicant was unable to identify the cause of this staining and states that repeated urinalyses have excluded urinary blood. An **OC** is raised to clarify whether presence of heme had been investigated, what the reason for the pronounced effect in MD animals is and regarding the possibility of a clinical relevance. In the same two studies, in which no prolongation of coagulation parameters were reported (studies R7834 and MRX-NC-004) no such staining of cage papers was reported.

Moderate decreases in body weight gains were seen repeatedly in males, whereas in females only at higher dosages.

An effect on liver were detected in rats (and to a lesser extent in dogs), as decreased liver weight, decreases in serum cholesterol, HDL, triglycerides but also changes in ALT/AST and proteins (albumin and globulins). It is considered plausible, that a change in hepatic activity with respect to bile acid and lipid metabolism potentially could affect related parameter i.e. cholesterol, HDL, triglyceride. However, the Applicant is asked to further elaborated on how changes in ALT/AST and proteins are related to the changed lipid metabolic activity in order to exclude a potential liver toxic effect of maralixibat (**OC**)**.**

Fecal bile acid excretion increased consistently already starting at the lowest doses tested (5 mg/kg/day; e.g. in study SA4865 at this dosage already up to 10-fold increase compared to control).

Slight increases of serum phosphorus were considered secondary to the changes in serum protein levels. Slightly increased serum sodium and/or chloride occurred at all levels and were attributed to possibly increased permeability of the intestinal tract following exposure to increased concentrations of bile acids.

Increasing weight of the intestinal segments in mice and rats was found and was accepted to be a result of poor absorption of large amounts of orally administered material. Increased urine calcium levels were attributed to cecal enlargement, which has been associated with increased intestinal absorption and renal excretion of calcium.

Microscopic changes in cecum, colon and rectum of mucus depletion of goblet cell, oedema in lamina propria, non-suppurative inflammation and mucosal epithelial alterations e.g. crowding of crypt cells with thickening of mucosa, was shown to be a result of increasing levels of luminal fBA. Even though increasing fBA levels is a pharmacological effect of maralixibat and most GI microscopical changes were reversed at treatment discontinuation, highlighting these changes is considered relevant, as abdominal pain and diarrhoea were observed as common adverse reactions in human patients. A potential risk of carcinogenic transformation of the GI mucosal epithelial alterations (e.g. crowding/proliferation of crypt cells) should be excluded histologically when 2-year rat CARC data are available (see **OC** in the CARC section).

In only two of the pivotal repeated dose toxicology studies in rats the in parallel treated toxicokinetics

groups included vehicle treated control group animals. In one of these studies (R7834M) the study documentation states that low concentrations of maralixibat (in the range of that one determined in maralixibat-treated low-dose group anmimals) were detected in control samples. An **OC** is raised in order to clarify the impact of contamination of toxicokinetics vehicle group samples with maralixibat on the validity of the whole set of study results obtained in this study and on possible contamination in other repeated dose toxicity studies.
Due to fatal bleeding events in HD animals in the GLP compliant pivotal repeated dose toxicity study in rats with the longest maralixibat treatment duration of 26 weeks (study SA4988), the dose of the MD animals is considered being the NOAEL, which is 150 mg/kg/day in male rats and 500 mg/kg/day in female rats. The exposure in male rats at this NOAEL was 5.42 ng/mL (C_{max}) and 108 ng•h/rnL (AUC) and in female animals 13.5 ng/mL (C_{max}) and 241 ng•h/mL (AUC). In humans after administration of once daily doses of 100 mg maralixibat for 14 days, mean C_{max} was 1.146 ng/mL and mean AUC_{0-24} was 4.614 ng•h/mL were reported. As this dosage in humans is about twice the clinically maximally intended one, assuming dose linearity, exposure based safety factors between NOAEL in this study and the maximum clinical dose of at least 9 (C_{max}, male animals, 150 mg/kg/day) and 47 (AUC male animals, 150 mg/kg/day) result. Considering that the main toxicological and dose limiting effect reported in this and the other repeated dose toxicity studies with maralixibat in rats is the prolongation of coagulation factors leading to bleeding events in higher doses and that this is an effect according to module 2.7.4 Summary Clinical Safety not observed in humans, these exposure based safety factors are considered sufficient. As the bioavailability of maralixibat is very low (in general below 1%) the use of doses instead of systemic exposure for calculation of safety margins is acceptable as well. According to a table provided by the Applicant, it appears that high safety margins to human doses exists, which is also the case in most of the studies conducted. However, it should be noted that safety margins were only calculated for selected repeat-dose studies in which a NOAEL was determined. In a number of the studies, no NOAEL could be determined. The primary toxicity observed in mice and rats, were prolongation of coagulation time. Changes in coagulation parameters were detected at doses as low as 5 mg/kg in rats (~HED = 0.8 mg/kg*), which do not provide much of a safety margin to the human dose of 0.532 mg/kg. Since the finding is within the clinically relevant dose range, it should be addressed in the SmPC (see **OC** below) but as also discussed the prolonged coagulation appears to be rodent specific and not a notable problem in dog, monkey or human patients.

Repeated dose toxicity studies of 2 weeks (EX4798), 4 weeks (SA4877, SA4945), 13 weeks (SA4991) and 6 to 12 month (SA4987) have been performed in beagle dogs. Maralixibat was given in an oral gelatin capsule up to 600 mg/kg/day (13 week study SA4991). With the exception of the 2 week study all studies were performed GLP compliant. All studies were accompanied by toxicokinetic evaluation. Recovery groups were included in the 4 week studies (SA4877, SA4945) and the 6/ 12 month study (SA4987). The data assessment of several studies is limited by obvious inaccuracies between study reports and the Toxicology written/tabulated summaries (studies: EX4798 and SA 4877) or in case of the 6/ 12 month study (SA4987) missing information/ study parts in the study report itself. These inaccuracies triggered one concern (please refer above).

In dogs, the tolerable dose was limited by exaggerated emesis as demonstrated in the 4 week study (SA4945). In this study, the high dose study group (600 mg/kg/day) was withdrawn due to exaggerated emesis. A maralixibat associated increase in emesis was observed at doses of 100 mg/kg/day and above. However, as dogs are prone to vomit and emesis was not listed as a common adverse reaction in humans in the SmPC section 4.8, the clinical relevance of the finding is considered low. In addition, in some cases slight decreases in body weights or body weight gains were observed. To a lesser extend as compared to rats, an increase in coagulation time was observed. Significant changes were observed in the combined 6/ 12 month repeated dose toxicity study at the highest dose group of 100 mg/kg/day. The applicant associates this finding with a vitamin K deficiency. The applicant tries indeed to present data on vitamin analysis obtained in study SA4987. However, the

respective sub-report is missing in the study report and the tabulated overview on the study results in the body report does not allow a clear assignment of dose groups to time points (**OC**/part 2).

Several other findings were associated with the pharmacodynamic activity such as a decreased plasma or liver cholesterol / HDL / triglyceride concentration or an increased fecal bile salt excretion, which are clearly caused by the pharmacodynamic activity of maralixibat. Some findings in the field of clinical chemistry such as total protein, albumin, globulin (and their respective ratio), [Cl^-] and [Na^+] concentration, which reached significance in the 6/ 12 month study in some dose groups are less clear associated with the pharmacodynamic activity but overall reversible and of low magnitude and, therefore, of low physiological relevance. Significant changes of liver enzyme concentrations in serum (increased aspartat aminotransferase, decreased alkaline phosphatase) in all dosing groups were considered also to be associated with the pharmacodynamic activity of maralixibat.

The applicant suggests that the combination of changes in serum phosphorus and ALP along with the increased urinary excretion of phosphorus suggests that decreased serum ALP values may be related to a decreased in osteoblastic activity. The conclusion appears to be reasonable, however, since the data presentation/analysis in the study report on urinary phosphorous excretion has to be considered currently incomplete (**OC**/part 1) this conclusion appears to be premature at this time point.

Two treatment-related histological changes (decreased eosinophilia in the fundic parietal cell and pyloric stomach cell cytoplasmic vacuolisation) were observed in the stomach at the Week 53 sacrifice. Decreased eosinophilia in the fundic parietal cell was noted in 100 mg/kg animals and pyloric stomach cell cytoplasmic vacuolisation was noted in 5, 20 and 100 mg/kg animals. These lesions remained present at the Week 61 (8-week reversal post 1 year of dosing) sacrifice. However, no explanation or comments on the clinical relevance of the findings were given. The Applicant is therefore asked to further elaborated on the changes and the clinical relevance **(OC)**.

The NOAEL in dogs based on the pivotal study with a duration of 1 year is 20 mg/kg/day. The applicant has presented several calculations of safety resulting in safety factors between 30 to 60 times. An approximation using exposure in humans and dogs would result in safety factors between 25 to 32 times. This is considered sufficient.

In addition, one non pivotal repeated dose toxicity study in cynomolgus monkey was submitted, which was performed more than 20 years ago. This study would be considered a breach of the principles of 3R nowadays. Furthermore, it should be noted that, the death of one monkey was caused by a dosing error (rupture of the oesophagus), which is hardly acceptable in non-human primates. The only noteworthy finding was an increased spleen weight and decreased colon weight.

A presentation of general toxicity findings in animals were currently not included in the SmPC section 5.3. A short summary of relevant findings (e.g. coagulation changes in mice/rats, emesis and changes in gastric cells in dogs) should be included with information on fold changes to clinically relevant dose or exposure (**OC**).

Genotoxicity and Carcinogenicity

Maralixibat was negative in *in vitro* Ames tests, chromosome aberration assays in CHO cells and in an oral *in vivo* micronucleus test performed in rats. Exposure was not determined in the Micronucleus test, however, there were signs of toxicity and excess mortality in the high dose animals indicating sufficient exposure of animals. Based on the provided studies, maralixibat is not considered to be genotoxic *in vitro* and *in vivo*.

Carcinogenicity was determined in rat and mouse carcinogenicity studies. One incomplete study evaluating carcinogenicity of maralixibat when administered to rats in the diet for at least 104 weeks

was provided. Due to deprioritization of the program by a previous sponsor, microscopic evaluation of tissues was discontinued and tissues were discarded, thus the full investigation of carcinogenicity potential from this study was not available. A 2 year rat carcinogenicity study is planned to be initiated in 4Q2020 and study results will be assessed when available. Based on the repeat-dose toxicity studies in rats, especially histopathological findings in the GI tract with evidence of initial proliferation or crowding of crypt cells is considered relevant to follow in a long-term development. Therefore, a potential risk of carcinogenic transformation of the GI mucosal epithelial alterations (e.g. crowding/proliferation of crypt cells) detected in the repeat-dose studies in rats should be excluded when 2-year rat carcinogenicity data are available **(OC)**. A further 2-year carcinogenicity in rats is planned by the sponsor and is anticipated to start in 4Q of 2020. The provided study design is considered adequate. Results of the planned carcinogenicity should be provided as soon as data become available (**OC**). In a 26-week oral carcinogenicity study in Tg rasH2 mice, there were higher incidences of bronchiolo-alveolar adenoma and carcinoma in males administered the high dose (25 mg/kg/d) maralixibat. Bronchiolo-alveolar adenomas were statistically significant for incidence and trend, as was the combined incidence of bronchiolo-alveolar adenoma and bronchiolo-alveolar carcinoma. The Applicant argues that lung tumours (bronchiolo-alveolar adenoma and carcinoma), are the most common spontaneous neoplasms reported in Tg rasH2 mice and their incidences in males administered 25 mg/kg/day maralixibat in this study (24% and 4%, respectively) are within published control ranges (Paranjpe et al., 2019). It is agreed that lung tumours are common background tumours in this mouse strain. However, Paranjpe et al reported a range for single adenoma of 0 - 24%/study (years 2004 – 2018) and an average of 8.98%. This indicates that lung adenoma incidences reported here are in the upper range of published background findings and higher than the average reported by Paranjpe et al. In order to judge if observed lung tumours incidences are only a background finding, the applicant should provide historical control data from the test facility were the study was performed (**OC**).

Reproductive and juvenile toxicity

In line with the proposed indication *"Treatment of progressive familial intrahepatic cholestasis type 2 in patients 1 year of age and older"*, a full range of reproductive and juvenile toxicity studies were submitted. Regarding the juvenile toxicity studies, the treatment period covers a human age range from birth to adolescence.

For the reproductive toxicity studies performed in rats, the sponsor selected dietary administration over gastric intubation (gavage) based on his experience with negative effects of aspiration of liquid formulations in previously conducted studies. However, it was shown later during drug development that gavage administration was principally possible even in juvenile rats. Unfortunately, no measure was taken to prevent access of offspring to the maternal diet in the PPND study, where adverse effects on pups' body weights were considered due to consuming the maternal diet at the time when the offspring start to eat on their own.

Toxicokinetic evaluations were done in almost all studies. The relevance of the values obtained can be questioned as the results were quite variable and contamination of control samples were observed in the PPND and juvenile toxicity studies. As contamination of control samples were also noted in other studies, this issue is addressed as OC in another part of this AR.

Toxic effects in adult rats generally resembled those observed in the repeated dose toxicity studies conducted in this species. While male fertility was not affected at all up to the highest doe tested (750 mg/kg/d), in female rats doses of 500 mg/kg/d and above reduced ovulation rate resulting in a decreased number of corpora lutea and implantation sites, respectively. Embryofetal development was not affected neither in rats nor in rabbits, despite maternal toxicity (body weight loss) in the latter

species. In addition, there were no adverse effects on prenatal and postnatal development including sexual maturation and reproductive capacity as well as learning and memory abilities noted in the offspring of dams exposed to maralixibat during gestation and lactation.

While treatment of juvenile rats following weaning did not adversely affect their viability, increased mortality / moribundity was noted in all dose groups in juvenile rats treated from PND 7 to 21. In the study report of the pivotal study, the high dose dose of 250 mg/kg/d was considered the MTD and calculation of exposure margins was based on this value set as the NOAEL in the nonclinical overview. This is not agreed, as in both the DRF and the pivotal study an increased mortality / moribundity was noted in young rats treated during PND7 to 21 and it is irrelevant here whether TK or main study animals were affected. In the pivotal study, five rats died or were euthanized in extremis in each dose group. While for the high dose group, septicemia was considered responsible for three deaths and gavage error for another one, the reason for the remaining deaths were unknown. Therefore, a NOAEL cannot be established and no exposure margin exists. For final assessment, the applicant is requested to comment in detail on the increased mortality / moribundity observed in the juvenile toxicity studies with treatment of pups from PND 7 to 21. In addition, bioavailability is different in juvenile rats from PND 7 to 21, with 17% bioavailability in contrast to < 1% as seen in other toxicity studies. The finding corresponds to literature data, showing that the intestine mucosal barrier in neonate rats can be penetrated by macromolecules more easily than in older individuals (Walthall et al, 2005). Rats are furthermore different from humans regarding its biliary system (rats have no gall bladder) and the human neonate starts life with a more mature gastrointestinal tract than neonatal rats. Since the Applicant argues that the use of maralixibat in children younger than 1 year old is supported by the absence of adverse effects in the PND7 to 21 juvenile toxicity study, a more detailed discussion of the translational value of the rat juvenile model with respect to the issues presented above should be provided (OC).

Local tolerance

Local tolerance was investigated in guinea pig on dermal sensitization potential after intradermal and topical administration and in rabbits on dermal and eye irritation. Potential local effects on colonic or rectal mucosa was investigated in female rats. The relevance of the studies in guinea pig on dermal sensitization potential after intradermal and topical administration and in rabbits on dermal and eye irritation appears to be of questionable. However, based on these studies a sensitization potential of maralixibat appears to be unlikely. In the eye, maralixibat showed a moderate to mild irritating potential. Potential local effects on colonic or rectal mucosa as found in the main toxicity studies were investigated in female rats further. In these studies a mucus depletion of colonic goblet cells, edema of the colonic lamina propria was confirmed. However, this finding was associated with the fasting conditions of the animals. The changes were not considered adverse by the Applicant but rather an effect of the pharmacological activity of maralixibat. It can be assumed, that these effects were caused by a local irritation caused by an increased bile salt concentration in the feces. The human relevance appears to be low.

Impurities

Potential genotoxic impurities were assessed in accordance with ICH M7(R1). Several compounds were identified as potential genotoxic impurities in maralixibat drug substance by *in silico* assessment using Derek Nexus and Leadscope Modell Apllier (LSMA) and expert review. Based on results of Ames assays several impurities are considered mutagenic and should be classified as Class 2 impurities. Several impurities were considered as Class 3 impurities based on *in silico* analyses and expert statements.

The Applicant classified several impurities as Class 3 impurities despite positive Ames test results. According to ICH M7(R1) compounds which are positive in bacterial mutagenicity assays should be formerlly classified as Class 2 compounds. Therefore, the applicant is requested to correctly classify impurities. Several impurities were classified as Class 5 impurities (non-mutagenic). The Applicant is requested to subject several impurities to Derek Nexus/LSMA analyses to exclude any mutagenic potential of these compounds .

3.2.4. Ecotoxicity/environmental risk assessment

An ERA Phase I for the active ingredient maralixibat chloride was provided. Based on prevalence data taken from the document on Public summary of opinion on orphan designation (EU/3/13/1216), Fpen was refined. The resulting PECsurface water of 0.004 µg/l does not exceed the action limit and the applicant concluded that Phase II testing is not triggered. Further PBT assessment has not been considered since the experimentally derived log Kow of 1.54 does not exceed the trigger value of 4.5.

The PECsurfacewater value for maralixibat chloride is below the action limit of 0.01 µg/L and the ERA can stop in Phase I. Maralixibat chloride is not a PBT substance as log Kow value does not exceed the action limit of 4.5.

Therefore, maralixibat chloride is not expected to pose a risk to the environment.

3.2.5. Discussion on non-clinical aspects

Although the development programme of maralixibat is in general in line with the requirements of ICH M3 several issues were identified which require response by the Applicant.

Maralixibat is considered a potent inhibitor of the human ASBT. In vitro proof of concept is considered established using H-14 cells transfected with the human ASBT. However, binding characteristics including potency of maralixibat and sequence homology of ASBT across non-clinical animal species used in in-vivo PD and toxicology studies was not presented. This should be clarified (**OC**).

Regarding pharmacology the only three molecules for which interaction of maralixibat was investigated in-vitro were the human ASBT, an alanine transporter and the hERG channel. No studies investigating secondary pharmacology have been submitted. The absence of secondary PD studies on targets outside the gastrointestinal system is considered acceptable, since the systemic exposure to maralixibat is negligible. However, the concentration in the gut, especially in the stomach, may be high and other transporters/enzymes/receptors than ASBT in the gut may be affected. In addition, data provided (alanine transporter only) is not considered sufficient for claiming high specificity of maralixibat for the ASBT. Applicant is asked to provide scientific argumentation on this concern and conducting a screen on receptors and other appropriate molecular structures in the gastrointestinal system including the stomach is recommended (**OC**).

In-vivo primary pharmacology data are in line with the ability of orally administered maralixibat to inhibit the re-uptake of bile acids and to increase the fecal excretion thereof. Nevertheless, several **OCs** were identified. The Applicant is e.g. asked to comment on the appropriateness of the partial bile duct ligation (PBDL) rat as an animal model considering the intended patient population (PFIC2), to comment on species differences in the size of the effects seen and relate the in-vitro determined IC_{50} to the estimated concentration on the ileal site of action.

The pharmacological effect of Maralixibat and UDCA was evaluated both separately and in combination. Beneficial effects were shown on excreted fBA and serum biomarkers for liver disease for both maralixibat, UDCA and the combination of the two compounds indicating that the effect could be

additive. No justification for choice of dose of UDCA (1 mg/kg/day) was presented. The human dose is 12-16 mg/kg/day (UDCA SPC). This should be clarified **(OC)**.

Although the hERG study was claimed to GLP compliant (MRX-NC-003), presence of maralixibat in test article formulations, could not be verified, even though the concentration was intended to be in the range of 200-700 ng/mL (0.3 – 1 µM). Since maralixibat is fully soluble in water and only sparingly soluble in organic solvents (3.2.S.1.3 General Properties, solubility in DMSO not presented), it seems peculiar that stock solutions were made in DMSO. The Applicant is asked to discuss and justify the validity of the submitted study (**OC**).

A core battery of safety pharmacology studies was presented using both oral and intravenous administration of maralixibat. The Applicant is asked to justify why the in-vivo safety pharmacology studies were not performed compliant to GLP regulations (**OC**). Otherwise, no sign for concerns were observed for cardiovascular, CNS or respiratory safety. However, co-administration of maralixibat with other medication used in the clinical setting was not discussed. These include oral bile acid binding resin, cholestyramine and rifampicin. Options to relieve pruritus include antihistamines, steroids and naltrexone (Clinical Overview). A discussion on potential implications of co-administering maralixibat with bile acid binding resin, cholestyramine and rifampicin should be presented **(OC)**.

The bioanalytical program appears to be and have been in good control with robust bioanalytical methods and documented stability of maralixibat in plasma using both heparin and EDTA as anticoagulants. From the final reports, some documents appear to be missing. Therefore, for completeness of the bioanalytical documentation, the validation report and amendment (KV95CJ and 12-8930(a)) supporting the most recent studies in adult and juvenile rat (MRX NC-001 and MRX-NC-004) should be submitted for assessment. Moreover, documentation of ISR in adult rat should be provided as well **(OC)**.

Pharmacokinetics of maralixibat was investigated in numerous studies in mice, adult and juvenile rats, pregnant and non-pregnant rats, pregnant rabbits, guinea pigs, dogs and monkeys. Studies on absorption, distribution, metabolism and excretion were provided. Maralixibat has to be characterized as a substance of very low bioavailability, with a local mode of action. Overall, the study program appears to be appropriate and complete, justifying from pharmacokinetic point of view the species involved in toxicity testing. However, several other concerns were raised. Noteworthy are contaminations of control groups and the implications caused by the adherence of maralixibat to laboratory appliances.

A very comprehensive general toxicity program was conducted in mice, rats, dogs and monkeys with maralixibat administration by oral gavage, dietary admix and capsules. Generally, bioavailability was low after oral administration (<1%) and the majority of findings in the toxicity studies were therefore related to the pharmacological effect of maralixibat rather than the systemic toxicity. Findings from iv administration in single-dose studies are therefore not likely to be clinically relevant.

Single dose toxicity studies in rats and dogs with oral and with IV administration did not raise safety concerns regarding the intended use in humans.

Repeated dose toxicity studies were performed in mice, rats, dogs and monkeys. Duration of maralixibat treatments in the GLP-compliant pivotal chronic repeated dose toxicity studies in rats and dogs comply with the requirements for the intended chronic use in humans.

General effects observed in most animal species, even at low doses, were increases in faecal bile acids (fBA) and changes in serum cholesterol, HDL, triglycerides, serum sodium/chloride; changes all considered a result of the pharmacologic activity of maralixibat. It was considered plausible, that a change in hepatic activity with respect to bile acid and lipid metabolism potentially could affect related

parameters i.e. cholesterol, HDL, triglyceride. However, it is more unclear how the observed changes in ALT/AST and proteins are related to the changed metabolic activity **(OC)**.

Prolonged coagulation time in rats with bleeding at higher doses and emesis in dogs were the primary toxicological findings from repeat-dose testing. Prolonged coagulation measured by reversable increases in PT and APTT values occurred at lower dose in male compared to female rats (detected at doses ≥5 mg/kg/day) and were considered a result of Vitamin K deficiency due to malabsorption secondary to the pharmacological effect of maralixibat. The changes appeared to be rodent specific and not a problem at clinically relevant doses in dog, monkey or human. Nevertheless, prolonged coagulation in rodents should be sufficiently described in the SmPC, especially since a narrow safety margin to clinically relevant doses exists (**OC**).

The appropriate wording of SmPC section 5.3 will depend on the responses of the Applicant given to the Other Concerns raised.

Maralixibat is not genotoxic. Concerning carcinogenicity testing of maralixibat, two Other Concerns were raised. A 2-year carcinogenicity in rats is planned and anticipated to start in 4Q of 2020. The provided study design is considered adequate. Results of the planned carcinogenicity should be provided as soon as data become available especially with view on histopathological findings in the GI tract with evidence of initial proliferation or crowding of crypt cells. In the 26-week carcinogenicity study in transgenic mice reported lung adenoma incidences are in the upper range of published background findings and higher than the average reported by Paranjpe et al. In order to judge if observed lung tumours incidences are only a background finding, the applicant should provide historical control data from the test facility were the study was performed.

Two Other Concerns were raised concerning genotoxic impurities. Three impurities should be classified as Class 2 impurities and the Applicant is requested to subject analyses to exclude any mutagenic potential of four other impurities.

3.2.6. Conclusion on non-clinical aspects

From non-clinical point of view there is no objection against granting of marketing authorisation provided that the responses to the Other Concerns raised is appropriate.

3.3. Clinical aspects

- ***Tabular overview of clinical studies***

The following tables give an overview on the different parts of the development programme. The first table refers to the PK and PD part of the programme:

Table 2 List of complete clinical studies informative for clinical pharmacology*:*

Study Number	Study Title
PK, ADME, and PD in Healthy Participants	
NB4-02-06-002	A Randomized, Double-Blind, Placebo-Controlled, Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Study of Ascending Single Oral Doses of SD-5613 in Healthy, Adult Subjects

Study Number	Study Title
NB4-02-06-003	A Randomized, Double-Blind, Placebo-Controlled, Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Study of Ascending Multiple Oral Doses of SD-5613 in Healthy Adult Subjects
NB4-02-06-004	A Pharmacokinetic Study of Single Oral Doses of [^{14}C]SD-5613 in Healthy Male Subjects
MRX-102	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
Studies in Pediatric Participants with Cholestatic Disease	
LUM001-301	ITCH: The Evaluation of the Intestinal Bile Acid Transport (IBAT) Inhibitor LUM001 in the Reduction of Pruritus in Alagille Syndrome, a Cholestatic Liver Disease
LUM001-302	IMAGO: 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 Paediatric Patients with Alagille Syndrome
LUM001-303	IMAGINE: 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
LUM001-304	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
LUM001-305	IMAGINE II: 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
LUM001-501	INDIGO STUDY: Open Label Study of the Efficacy and Long Term Safety of LUM001, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi), in the Treatment of Cholestatic Liver Disease in Pediatric Patients with Progressive Familial Intrahepatic Cholestasis
Studies in Other Populations	
SHP625-101	A Randomized, Blinded, Placebo-controlled, Phase 1 Study to Assess the Relative Potency of Multiple Oral Doses of LUM001 and SHP626 in Overweight and Obese Adult Subjects as Assessed by Fecal Bile Acid Excretion
NB4-02-06-014	Clinical Study Report for a Randomized, Double-Blind. Placebo-Controlled, Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Study of Ascending Multiple, Oral Doses of SD-5613 in Adolescent Subjects with Hypercholesterolemia
LUM001-401	CAMEO Study: A Pilot, Open-label Study to Evaluate the Safety, Tolerability and Efficacy of LUM001, an Apical Sodium-dependent Bile Acid Transporter Inhibitor (ASBTi), in Patients with Primary Sclerosing Cholangitis
Clinical Drug-Drug PK Interaction Studies	
NB4-02-06-008	A Randomized, Double-Blind Study Comparing SD-5613/Statin Combination Therapy and Statin Monotherapy in Healthy, Adult Subjects
NB4-01-06-019	Assessment of Pharmacokinetic Drug-Drug Interaction Between SD-5613, Simvastatin and Lovastatin After Oral Administration of Multiple Concomitant Doses in Healthy Volunteers
NB4-02-06-020	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
LUM001-201	A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate LUM001, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi), in Combination with Ursodeoxycholic Acid (UDCA) in Patients with Primary Biliary Cirrhosis

Abbreviations: PD = pharmacodynamics; PK = pharmacokinetics

The PK programme also included "in-silico" studies, which are displayed in the following table:

Table 3 List of in-silico studies on pharmacology of maralixibat.

Study Number	Study Title
MRX-NC-007	Development and Verification of a PBPK Model for Maralixibat Oral Dosing and Its Application to Predict the Drug-Drug Interaction Potential for Competitive and Time-Dependent Inhibition of CYP3A4 Enzyme in Adult Healthy Human Volunteers
MRX-NC-011	Exploratory Dose-Response Analysis of Maralixibat and Serum Bile Acid

Clinical studies in the targeted indication are presented in section 3.3.5.

Note: The doses described in section 3.3 are of maralixibat chloride but are presented as "maralixibat". For example, doses of 140, 280, and 560 µg/kg maralixibat chloride are equivalent to 133, 266, and 532 µg/kg maralixibat free base, respectively, but will be referred to as 140, 280, and 560 µg/kg maralixibat in line with the original documentation submitted.

3.3.1. Pharmacokinetics

Methods

Plasma concentrations of maralixibat were determined by validated LC-MS/MS methods. Serum levels of conjugated and unconjugated bile acids were also determined using validated LC-MS/MS methods as well as concentrations of the endogenous biomarker, 7α-hydroxy-cholest-4-en-3-one (C4). The method validations and documentation for sample analysis is accepted even some information is missing. The missing information is not considered critical since the systemic exposure of maralixibat is very low.

Additional validation results for a couple of biomarkers have been presented, of which the one for bile acids is considered the most important, because this has been used both as PD, as well as efficacy parameter. There is a remaining concern on the linearity of the method which should be clarified **(OC)**. Also, there were no validation studies presented for the biomarkers FGF-19, FGF-21, autotaxin and 7α-Hydroxy-4-cholesten-3-one, which need to be shown **(OC)**. Because for the determination of "routine" liver parameters in children different assays were used in different regions of the world, it is currently also unclear whether these have been adequately cross-validated, and whether results from these regions is therefore comparable. The applicant is requested to present such data **(OC)**.

Absorption

The applicant has conducted 4 PK studies in order to determine the PK of the compound in healthy adults subjects

- Study MN4-02-06-002 which has investigated the safety and PK of single doses
- Study NB402-06-003, which has investigated multiple (ascending doses)
- Study NB-02-06-004 which was an ADME study with radioactively marked compound given as single dose
- Study MRX-102 which was a single to study with the to-be-marketed liquid formulation.

Despite a sensitive method used for the detection of the compound (LloQ=0.025 ng/ml) hardly any substance could be found in plasma or could derived PK parameters be calculated in appropriate manner. In consequence, it is concluded that the substances is very poorly absorbed, and maximum plasma levels are reached between 1.0 to 3.0 hours. The estimated half life of the compound in plasma has been determined to be about 4 hours, but this had to be based on the evaluation of high doses (above the therapeutic dose-range proposed for children with PFIC2). The study with the to-be-marketed formulation did not provide different results.

The following table shows "typical" results from these early studies, demonstrating the negligible absorption:

Table 4 Summary of Pharmacokinetic Parameters of Plasma Maralixibat (Single Ascending Doses) (Study NB4-02-06-002):

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]

In accordance with this, the ADME study did not detect measurable radioactivity in plasma, and more than 99% of the detected radioactivity was detected in faeces, primarily as parent compound (>94%), which three different metabolites having a share of 1%-5%. The radioactivity deteted in urine amounted to 0.066%. Although the overall recovery in this study was only about 73%, the results were considered valid, due to the fact that they were in accordance with the previous studies in healthy volunteers showing minimal plasma levels.

In the pre-clinical study MRXNC-001, an immense difference in the exposure of maralixibat between PND 7 and PND 20 juvenile rats was observed. An explanation for this could be a high degree of maturation of the GI tract as the animals grow older. The Applicant is asked to discuss how to interpret this result compared to the maturation of the human GI tract **(OC).**

Bioavailability/Bioequivalence

Data on absolute bioavailability are, however, not available because no i.v. administration was included in any of the studies. The estimated bioavailability is expected to be less than 1%. The applicant has therefore also not evaluated bioequivalence of the different formulations, which is also based on the fact that the solid dosage forms used were rapidly dissolving pharmaceutical forms, which is altogether considered acceptable.

Influence of food

The applicant has evaluated the influence of food in two studies (Study MRX-102 and Study NB4-02-06-002) with different dose levels (10, 20, and 45 mg). These studies were partially hampered by the low plasma levels detected. Based on the available results, however, it could be concluded that food further reduces the overall low bioavailability of the compound to a relevant extent (60-80%).

The results for the statistical evaluation of study MRX-201 are shown in in the following table:

Table 5 Statistical Analysis of Food Effect (Study MRX-102)

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	AUClast (ng•h/mL)	8	0.51	3.60	14.2	5.80, 34.69
	Cmax (ng/mL)	8	0.45	1.69	26.8	20.22, 35.56
Maralixibat 45 mg	AUClast (ng•h/mL)	9	1.39	4.53	30.7	18.16, 51.92
	Cmax (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: MRX-102, Table 14.2.1.3

While in a compound with systemic action, this would lead to the recommendation to administer the compound in the fasted state, the proposed recommendation is to administer the compound half an hour before food intake is obviously based on theoretical reflections based on PK as well as PD considerations: The highest concentrations would need to be present in the intestine (lower part of the small intestine, which is the location of (re)-absorption of bile acids), therefore, any absorption would be regarded to be untoward with regard to the PD effects, as well as with regard to safety of the compound. While optimality of the proposed 30 minute window has not been investigated, it takes not only the food effect into account, but also the consideration that inhibition of bile-acid (re-)absorption would be inhibited highest at the time of highest bile acid secretion, which itself depends on the intake of food. However, for the children and their caregivers, administration together with food would be more feasible and this should be discussed (OC).

Distribution

The compound – once absorbed, is expected to be highly bound to plasma proteins (>90% in vitro) based on the results of the in-vitro study M3099225. Plasma protein binding was within 84.2% to 97.3% for all species tested and was found to be concentration independent across the concentration range. Protein binding, as well as the distribution of the compound have not been investigated in vivo. While this could be acceptable (distribution might not be estimated considering the incomplete PK profiles), the applicant has not included a justification for this omission, and this should be addressed **(OC)**.

Elimination

As seen in the ADME study, the compound is mainly eliminated in the faeces, and hepatic uptake, metabolism and/or renal excretion are not expected to play a relevant role in PK. Approximately 72% of the radioactive dose was detected in feces compared to <1% in urine.

In 10 healthy fasted subjects (study MRX-102) receiving single doses of 100 mg maralixibat, the estimated terminal half-life (t1/2) was 1,97 h. The estimated clearance of 7700 L/h is unreliable with the extrapolation portion of AUC_{∞} >20% for the majority of participants.

In patients, no elimination parameters could be calculated.

In consequence of the low plasma levels, the applicant has investigated the potential for hepatic metabolism in vitro only. The metabolic profile of the compound has been determined in-vitro with study M4099002, and it was shown that metabolisation appears to be extensive (almost 70% with 60 minute incubation).More than 10 metabolites were identified (of which none was unique to humans) However, in face of the minimal absorption, as well as less than 3% of radioactivity detected as metabolites (most of these in faeces), the relevance of the findings appears to be minor.

Although extensive metabolism of the compound was detected and 6 metabolites have been characterised, no further investigation was performed due to the expected small contribution of metabolites to the overall limited total exposure. This is considered acceptable.

Dose proportionality

Dose-proportionality as a method of PK characterisation was also hampered by the fact that the compound is poorly absorbed only. There was indication of increasing concentration/exposure with doses higher than 20 mg, but a clear linear relation could not be demonstrated. No relevant differences were detected between single and multiple doses of the compound, although a formal evaluation of time-dependency was not conducted.

Intra- and inter-individual variability

As expected, no numerical determination of the variability of PK parameters was presented, which is, however, also considered acceptable. A high variability of PK parameters is obvious from the data. In the 100 mg dosing group of the food effect study MRX-102, the coefficient of variation for AUC0-inf and Cmax is 73% and 52%, respectively.

Special populations

The applicant has investigated PK in different diseased populations (adults with cholestatic liver diseases, such as PBC and PSC), adolescents with hypercholesterolaemia, and children with Alagille and PFIC. All these investigations were done with sparse sampling, both after single and multiple drug administration, with the time point mainly after 4 hours post drug intake. The doses used in these studies were variable, but usually (based on body weight) lower than those proposed for the PFIC2 target population. In all these studies, drug concentrations were below LoQ in the majority of patients, and hardly any concentrations were detected being above 1.0 ng/ml. This also clearly applies to the study in the target population where the doses proposed for marketing were tested.

The applicant has not evaluated the PK in special populations, such as renal or hepatically impaired. While this could be acceptable considering the PK properties with poor absorption, the applicant has not provided adequate argumentation, why such studies would not be needed, and has not investigated (e.g. by modelling) how plasma concentrations could develop when severe hepatic or renal impairment is present. In addition, adequate statements with regard to the intake of the compound in patients with hepatic or renal impairment are currently not proposed in the product information, which is not acceptable **(OC).** Due to the fact that PFIC patients may at some point of time suffer from hepatic impairment, such argumentation should be provided. Also, because some patients showed relevantly higher concentrations in comparison to mean values, the applicant should evaluate whether any factors for "increased" plasma concentrations can be identified **(OC)**. This also relates to the fact that, owing to the low plasma levels, the applicant did also not investigate whether demographic characteristics (race, weight, gender etc.) have a relevant effect on PK. As indicated above, the evaluation of potential factors for "high" plasma concentrations, including these demographic factors should be undertaken (**OC)**.

Interactions

The applicant has determined the interaction potential of the compound mainly with in-vitro investigations, using in-vitro assays for cytochrome induction, and inhibition, as well as transporter inhibitions. These investigations identified CYP3A4/5 and the transporter OATP2B1 as the only "candidates" for a relevant inhibition by the compound. This is not only based on the (relative to the other CYPs and transporters) lower inhibitory concentrations, but also to the fact that both are also located in the intestinal mucosa, and could cause a PK inhibition at the local, pre-systemic level.

The applicant is presenting 4 in-vivo studies in order to address the potential for drug-drug interaction. Three of these studies (NB4-02-06-008, NB4-01-06-019, and NB4-02-06-020) were studies with the statins lovastatin, simvastatin, and atorvastatin. These compounds were thought to be substrates of several organic anion transporters, but it is unclear whether this also concerns OATP2B1. At least two of the compounds are also highly dependent on CYP3A4 metabolism (simvastatin and atorvastatin). The statistical results for the study conducted with atorvastatin are displayed in the following table:

Table 6 Results of a Statistical Comparison of Atorvastatin,ortho-Hydroxyatorvastatin and para-Hydroxyatorvastatin Pharmacokinetic Parameters Following Morning (AM) Administration of Atorvastatin Alone or in Combination with SD-5613

Analyte	Parameter	Least Squares Means [a]				Comparison: Test/Reference [b]		
		Test [c]		Reference [c]				
		N	Mean	N	Mean	Ratio	90% CI	P-value
Atorvastatin	AUC_{0-24} (hr*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_{0-24} (hr*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-hydroxyatorvastatin	AUC_{0-24} (hr*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

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 original scale.
[b] Ratio of least squares means and 90% confidence interval 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).
Source: Tables T8.1 to T8.3.

These studies did not detect a potential for a clinically relevant drug-drug interaction. However, all these studies were hampered by the fact that only rather small doses of maralixibat were used, and the applicant would therefore need to further address the issue with regard to both the potential for local interaction on CYP3A4 as well as OATP2B1 **(OC).**

The applicant has further evaluated the interaction potential with regard to CYP3A4 with an in-silico study:

The developed PBPK-Model indicates that CYP3A4 inhibition potential is likely low due to low systemic exposure. Modeled population mean increase in AUC and Cmax after the highest dose of 600 mg/kg BID was 10%, calculated with the predicted fuGut of 0.096. In a worst case scenario calculated for a fuGut of 1, the increase of midazolam exposure was estimated to be 31%. Small number of subjects, large variability and many measurements near the LOQ prevented further model refinement. Some documents of this study appear to be missing (e.g. Appendix A section 7, and goodness-of-fit-plots should also be submitted **(OC).** Sensitivity analysis varying the parameters 10-fold each side showed an increase of midazolam exposure of 40% which might be clinically relevant for DDI regarding CYP3A4. Model development was impaired by the low number of individuals (6 subjects) contributing to model verification. Additionally, model development was based on data collected in healthy adults, transferability to a pediatric population with cholestatic disease is regarded as limited.

Formally, a further study was presented as interaction study, which evaluated the potential for interaction with ursodeoxycholic acid, which is the standard therapy in patients with PBC, in which population this study was conducted as a phase 2 study. With regard to the influence of maralixibat on UDCA kinetics, only inconclusive results were obtained. This study is characterised rather as a PD interaction study (maralixibat potentially preventing absorption of UDCA), rather than a PK interaction study (see below).

In conclusion, while the potential for PK interactions appears also to be low based on the low plasma concentrations, a full elucidation of the interaction potential on the local level of intestine has not been presented and could need further evaluation.

3.3.2. Pharmacodynamics

For the mechanism of action of the compound, see also Chapter 2.2.

The evaluation of the PD of the compound is mainly based on the induced changes in levels of (fasting) serum bile acids, as well as the content of bile acids in faeces. Further biomarkers were also explored involved in the reactive changes of bile acid synthesis.

The molecule is an inhibitor of the apical sodium bile-acid transporter, a transmembrane protein localised on the luminal surface of ileal enterocytes (terminal 25% of the small intestine) which mediates the uptake of conjugated bile acids across the brush border membrane of the enterocyte. The IC50 with regard to IBAT inhibition has been determined a 0.3 nM.

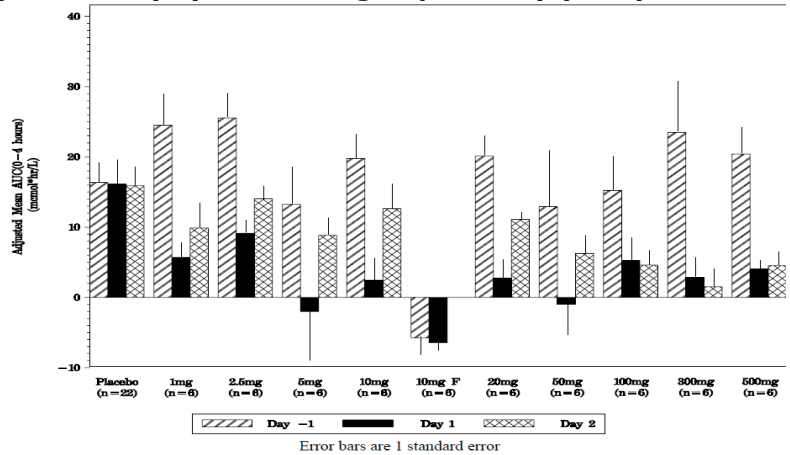
Pharmacodynamics in Healthy Adult Participants

Pharmacodynamic effects in humans were already tested in non-diseased subjects in the early studies NB4-02-06-002, NB-02-06-003, and also in Study SHP625-101, which was conducted in obese, but otherwise healthy subjects.

In healthy subjects, a decrease of serum bile acids was seen, which appeared to be dose-dependent with modest correlation and consistent only at doses higher than 2.5-5 mg. The missing clear correlation to the doses administered could potentially be explained by the high influence of food intake. An increase of faecal bile acid secretion was also detected in a more clear dose-dependent manner, and was detected at all doses administered across studies, and without relevant differences between single and multiple administrations, however with high variability between subjects. In addition, a consistent increase in faecal weight was seen, with small increases and a tendency for increased number of bowel movements and liquid stool consistency. Serum 7αC4 concentration as a parameter for increase in bile acid synthesis was increased. In healthy subjects, changes in lipid parameters were rather modest, but detectable (e.g. LDL decrease). The studies do provide a clear proof of the concept of reducing serum bile acids by inhibiting the reabsorption within the endogenous bile acid recycling.

In study NB4-02-06-002, a total of 82 subjects received single oral doses of maralixibat ranging from 1 mg to 500 mg or placebo. Profiles of sBA and fBA prior to and following dosing with maralixibat or placebo served as pharmacodynamic markers. The changes of bile acides serum concentrations are shown in the following figure.

Figure 4 Baseline adjusted serum total bile acids concentation: Mean AUC (0-4 hours; µmoloxhr/l) by treatment group and day (Study NB4-02-06-002)



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 802 (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.

In study NB4-02-06-003, a total of 167 subjects were treated for 28 days receiving multiple doses of maralixibat ranging from 0.5 mg to 100 mg, or placebo. Profiles of sBA and fBA following dosing with maralixibat or placebo served as pharmacodynamic markers. sBA levels were assessed on Days 1 and 14, and similar to the single ascending dose data, suppression of basal sBA as well as postprandial increases was observed on Day 1. Suppression of sBA increased with increasing doses. In contrast, on Day 14, the effects of higher doses appeared to be attenuated (pooled 10 to 100 mg doses showed greater suppression on Day 1 vs. pooled 1 to 5 mg doses but this was not apparent on Day 14

Table 7 Summary of Daily Total Faecal Bile Acids Excretion (Multiple Ascending Doses of Maralixibat) (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.

In study SHP625-101, the pharmacodynamics of maralixibat was assessed in overweight and obese participants (body weight > 63.5 kg and mean body weight 91 kg). Maralixibat at doses of 10 mg once daily, 20 mg once daily, 50 mg once daily, 100 mg once daily or 50 mg twice daily, or matching placebo, was administered for 7 consecutive days. The primary endpoint was fBA; sBA was included as a secondary endpoint. Mean fBA change from baseline increased in all participants who received maralixibat and increased with increasing total daily dose. The greatest mean change from baseline in

total sBA concentration at Day 7 was an increase of 2.571 (2.3099) ng/mL observed in subjects who received placebo. In contrast, no significant change in mean sBA from baseline was demonstrated in this population.

Pharmacodynamics in Paediatric Participants with Cholestatic Disease

PD properties were further evaluated in studies with different disease as the target population, with study NB4-02-06-014 conducted in adolescents with hypercholesterolaemia, study LUM001-401 in patients with PSC, study LUM001-201 in adult patients with PBC, and in various studies in children suffering from Alagille Syndrome (LUM001-301 to LUM001-305).

For patient populations with "modest" cholestasis only, such as patients suffering from PBC or PSC, rather modest decreases of serum bile acids were detected, but in both populations effects were also seen with regard to a reduction of pruritic symptoms, and increase in 7-alpha-C4, as well as for a reduction of LDL-cholesterol. Because these studies were also designed as phase 2 studies, the disease-specific parameters (biomarkers) such as bilirubin and ALP (as well as transaminases) were also investigated, but no relevant effects on these could be detected.

In study NB4-02-06-014 serum bile acids were also partly reduced in adolescent patients with hypercholesterolaemia, although some inconsistencies were found, obviously due to the low doses administered (highest dose 5 mg). There was a clear tendency for lowering of LDL-C, except in the lowest dose group (0.1 mg)

Further studies have been conducted with the compound in patients with hypercholesterolemia, evaluating mainly the effects on lipid parameters. In one of these studies (Study NB4-01-02-035-ASR) pharmacodynamic effects of the compound with regard to bile acid reduction and serum lipids in different diet regimens has been investigated. It could be shown that PD activity increases with increasing caloric content, as well as with increasing fat content, both for (most of) the serum lipids, as well as for serum bile acids. Further studies in this patient population (BATAHC-0524-037 and 038) have investigated whether a "sustained release" mimicking intake of small doses distributed over the day would (in order to assure a more constant blocking of the bile acid transporter) be able to deliver higher changes in serum bile acids and lipid parameters. This was obviously not the case in these studies, which can be considered relevant for the proposed once daily dosing also proposed for the PFIC2 population. However, transferability from this study might be questioned, and the applicant would need to justify the proposed dosing schedule further (OC). Finally, in this population, a full factorial design study was conducted in order to see whether combination treatment with statins would make sense. In this study, while modest effects with monotherapy with maralixibat on LDL-C were detected, there was no additional effect when given with atorvastatin.

The 5 studies presented in children with Alagille Syndrome (LUM001-301, LUM001-302, LUM001-303, LUM001-304, LUM001-305), in which doses at quite similar levels as proposed for the target population were administered, showed also a clear decrease of serum bile acid concentrations, however, with some fluctuations between doses and over time. However, a clear superiority to placebo with regard to this parameter could be demonstrated within these studies. The studies in Alagille's have shown consistent results across different studies, of which 3 were placebo-controlled, and of which at least one has shown clearly clinically relevant, and statistically significant results with regard to the reduction in serum bile acids. Two of the studies have shown more variable effects, without a clear dose response, but many of the difficulties to demonstrate statistically significant differences could be attributed to small sample size, potential high variability in baseline sBA values, and high fluctuation during the course of the disease. The long-term extension studies have demonstrated that the reduction of sBAs can be maintained over time, with further potential reductions to be achieved in long-term, up to 244 weeks.

Pharmacodynamics in the target population:

One study (Study LUM001-501) is presented for the target population PFIC2, which is presented as the pivotal study (see efficacy assessment). In this study, the primary outcome parameter was serum bile acid concentrations over time. Relevant reductions of sBAs could also be shown for this population, which, however appeared to be somewhat smaller in magnitude as compared to the Alagille's population. For the further assessment of this study, it is, however, referred to the chapter on efficacy.

Secondary pharmacology

The applicant has not conducted any dedicated studies on the potential for effects base on secondary pharmacology. The applicant, however, indicates that e.g. the QT prolongation potential has been evaluated and presents data from the food interaction study MRX-102 which has extensively recorded and analysed ECG data. Study MRX-102 appears to include sufficient data to exclude a potential for QT prolongation, when considered together with the available pre-clinical information, and the safety margins calculated.

PD interactions

The applicant has not conducted any PD interaction studies. However, the study as above with atorvastatin (NB4-00-02-006), as well as the study in patients with PBC (LUM001-201) could also be regarded to represent PD interaction studies.

For atorvastatin, no additional effects on plasma lipids were detected (as seen above, and obviously no indication for PD interaction exists).

For the study in PBC patients, the potential PK interaction was evaluated, but did not yield conclusive results, potentially due to an incomplete evaluation of UDCA (no consideration of endogenous UDCA conjugated and unconjugated UDCA). In similar way, no conclusions appear to be possible when looked at the PD parameters ALP, bilirubin, and liver transaminases. Whether the potential for UDCA interaction has been sufficiently evaluated, however, appears to be questionable, due to the fact that it might also be relevant for the target population, in as many patients with PFIC are treated with UDCA. Whether the potential interaction can be classified as PK or PD interaction appears to be semantics only, however, whether inhibition of the bile acid transporter would also inhibit (re)absorption of UDCA could be clinically relevant also in the target population. The applicant is requested to address this concern **(OC).**

In conclusion, the PD properties of the compound have been sufficiently investigated, and adequately characterised. By inhibiting bile acid absorption, the compound increases the faecal excretion of bile acids, thereby inducing the potential for gastrointestinal effects (increase stool weight, increased stool frequency and diarrhoea). By blocking (re-)absorption of endogenous bile acids, the compound is able to reduce serum bile acids in healthy subjects, as well as in a variety of disease states, including the severe cholestatic childhood diseases PFIC2 and Alagille's syndrome. The reduction of the endogenous bile acid pool induces an obvious increase in the production of bile acids, as measured by appropriate biomarkers in healthy volunteers. This mechanism, however, is not considered relevant in patients with highly pathological serum bile acid levels where levels below normal are not achieved. In addition, the compound induces a modest improvement in serum lipid parameters (e.g. increase in LDL-C) which might be based on both the bile acid sequestrant effects, as well as the reactive increase of bile acid production. However, these effects appear not relevant for the target population of PFIC2 but is nevertheless considered reassuring.

3.3.3. Discussion on clinical pharmacology

The present MAA concerns maralixibat chloride (hereafter maralixibat), an oral inhibitor of the apical sodium-dependent bile acid transporter (ASBT) for the treatment of progressive familial intrahepatic cholestasis type 2 (PFIC2), a rare cholestatic liver disease in children. Maralixibat is a selective ASBT inhibitor small molecule with limited systemic exposure and a molecular weight of 710 Da and harbouring a positively charged quaternary nitrogen atom. Maralixibat inhibits BA reabsorption, thereby increasing fecal bile acid (fBA) excretion and lowering serum bile acid (sBA) levels. The recommended dose is 266 µg/kg of maralixibat once or twice daily, which corresponds to 280 µg/kg maralixibat chloride (the substance as a salt).

The investigations with regard to clinical pharmacology of the compound reflect two basic facts on the compound:

- Maralixibat is very poorly absorbed, and measurable plasma levels are only observed in a minority of subjects, both in healthy subjects, as well as in a variety of disease states
- Maralixibat has a long history of development with multiple changes of sponsors, and multiple changes of the envisaged target populations. This is reflected in a variety of (diseased) populations included not only in the PK, but also in the PD investigations (with some of the studies presented as PD studies, originally intended as early (Phase 2) development studies.

These facts explain to a relevant part the scope and extent of the studies conducted.

While for PK, already early in the development, it was clear that the compound hardly develops measurable plasma concentrations and may therefore need only a restricted investigation of the overall PK, the applicant has not only omitted the conduct of several studies addressing specific properties, but also has omitted addressing factors of the characterisation of PK, such as volume of distribution, the influence of demographic characteristics on PK, and PD properties with regard to interactions at the local level in the GI tract (with respect to enzymes and transporters playing a role in transmembrane transport or metabolisation) and interactions potentially affecting the target population (e.g. fat-soluble vitamins, UDCA).

No dedicated formal dose finding studies in the target population have been performed. It is unclear how exactly the dosing schedule has arisen, both with regard to the proposed once daily dosing (regular dose), as well as with regard to the weight-based dosing, which was only introduced when investigating children in the ALGS and PFIC2 studies. While weight-based dosing in younger children with PFIC2 could be acceptable, as already tested in the clinical study (LUM001-501), the question arises whether it is also adequate in adolescents and adults. The Applicant should elaborate on and justify the creation of the proposed dosing regimen (**OC)**.

On the other hand, the low levels of plasma concentrations do indeed provide a high level of reassurance that the compound may be largely devoid of systemic (off-target) actions and some of the missing information is not considered critical. This is reflected in the results of all investigations conducted both in healthy volunteers, as well as in patients.

The applicant has thoroughly investigated the primary pharmacodynamic targets of IBAT inhibition, which extends from bile acid sequestration (increase in content of bile acids in the faeces) and a reduction of serum bile acids (which only become obvious at higher doses in healthy subjects) to the further consequences of this primary action: increase in faecal weight, stool frequency, and potentially diarrhoea at the local level, induction of bile-acid synthesis in healthy volunteers (as measured by the biomarker 7-alpha-C4), and a modest improvement of the serum lipid profile (LDL reduction, HDL increase, triglyceride decrease). The induction of bile acid synthesis could regularly be detected in patients with normal levels of serum bile acids by increases in 7-alpha-C4, and by a consequential

decrease of FGF-19 and FGF-21. However, in patients with cholestatic disease, this has not always been observed, or has not been investigated in all studies conducted. The potential for increased bile acid production (and its related biomarkers) appears therefore to be dependent on the normalisation or "sub-normalisation" of serum bile acids, which is, however, not regularly achieved with the compound in patients with high serum bile acids, such as those suffering from Alagille-Syndrome or PFIC.

Although the potential for drug-drug interaction appears to be low, and systemic interactions seem to be very unlikely, or have adequately been investigated (and excluded) the applicant has not fully addressed the potential for local interactions in the gastrointestinal tract with regard to potential concomitant medication (including potential interaction with fat-soluble vitamins) and at relevant doses administered.

3.3.4. Conclusions on clinical pharmacology

The clinical pharmacology package consists of a large number of studies. Due to the low absorption of maralixibat, PK parameters were not calculable with the doses employed in paediatric patients. The basic PK and PD properties of the compound have been adequately characterised, however, there are several aspects, both from the PK as well as the PD level that need additional consideration.

3.3.5. Clinical efficacy

Key evidence on efficacy is provided from the pivotal Study LUM001-501. Supportive evidence is derived from the comparative analysis of data from the pivotal study and selected natural history control from the global natural history data registry, NAPPED. Further studies, MRX-502 (DB, placebo-controlled and randomised study) and MRX-503 (extension), are currently enrolling. An additional ongoing study MRX-800 is an open-label extension study which enrolled the patients from LUM001-501. No data are available from the ongoing studies. In total efficacy database in PFIC2 is limited to 25 patients of 1-13 years of age.

Maralixibat is also being tested in Alagille syndrome (ALGS). This condition is also associated with increased levels of bile acid and pruritus. However, pathophysiology of ALGS and PFIC2 differ and efficacy data collected in ALGS are not considered in this assessment.

Details to the presented clinical efficacy studies are provided in the table below.

Table 8 Overview of the completed and ongoing clinical studies relevant to efficacy in the targeted indication

Study ID	Locations (# Study Centers)	Study Start Total Enrollment Status	Design Control Type	Study and Control Drug Route & Regimen	Study Objective	# Participants by Arm Entered	Duration	Gender M/F Median Age (Range)	Diagnosis Inclusion Criteria	Primary Efficacy Endpoint
LUM001-501	France Poland UK US (10)	Start: 12 Feb 2014 Enrolled N=33 Completed	Phase 2, open-label, multiple-dose study	Maralixibat 280 µg/kg QD 280 µg/kg BID for non responders during (optional) follow-up treatment	Long-term safety and efficacy	MRX: 33 -8 PFIC1 -25 PFIC2: 19 nt-PFIC2 and 6 t-PFIC2	72-week observation period optional treatment extension	14 M 19 F Median Age (Range): 3.0 (1-13)	PFIC sBA > 3 x ULN No prior surgical treatment	Fasting sBA change from baseline to Week 13/ ET Post-hoc sBA responder analysis
LUM001-501/ Natural history comparison	-	Completed	Retrospective comparison	-	Comparison of "hard clinical endpoints"	MRX: 25 (19 nt-PFIC2, 6 t-PFIC2) NAPPED: 98 (72 nt-PFIC2, 26 t-PFIC2)	-	PFIC2 MRX: 25 8 M, 17 F NAPPED: 98 43 M, 51 F, 4 unknown nt-PFIC2 MRX: 19 6 M, 13 F NAPPED: 72 31 M, 38 F, 3 unknown	PFIC2 sBA > 3 x ULN No prior surgical treatment	EFS

EFS = event-free survival; ET = end of treatment; F = female; ID = identification; M = male; MRX = maralixibat; N = number of participants; PFIC = progressive familial intrahepatic cholestasis; sBA = serum bile acid; UK = United Kingdom; US = United States.

Dose-response studies and main clinical studies

No dedicated dose-finding study in the target population with maralixibat has been conducted and dosing recommendations are based primarily on the experience with maralixibat in the patients with PFIC2 in LUM001-501 (pivotal) study, as well as, in healthy adult volunteers and adult patients with other indications. The therapeutic dose of 280 µg/kg/day QD maralixibat chloride (equivalent to 266 µg/kg/day maralixibat free base), taken approximately 30 minutes before the first meal of the day, with the option of dose increase to 280 µg/kg BID (532 µg/kg/day maralixibat free base equivalent to 560 µg/kg/day maralixibat chloride), with the second daily dose taken approximately 30 minutes before the evening meal is proposed. Both doses 280 µg/kg/day (266 µg/kg free base QD; in 25 patients) and 560 µg/kg/day (given as 266 µg/kg free base BID in 5 patients) were tested in the targeted population in the pivotal study.

Dose titration is not being recommended based on the experience with maralixibat in non-target population (adult healthy subjects and patients with hypercholesterolaemia). Majority of the AEs were GI-related mostly mild and transient events, assumed to be related to increased bile acid (BA) excretion and presence in the lower colon in response to ASBT inhibition. Scientific hypothesis is that maralixibat will produce smaller increase in free BAs in the lower colon in the patients with PFIC2 due to the limited bile flow compared to healthy volunteers and hypercholesterolemic patients. Consequently, better tolerability is hypothesised in the PFIC2 population and the starting dose of 280 µg/kg/day is assumed to be appropriate.

Twice daily dosing is proposed for the maximum dose (i.e. 560 µg/kg/day) based on the findings of a healthy volunteers study in adult males (Study SHP625-101), which evaluated QD and BID dosing regimens (up to 100 mg QD and 50 mg BID). It is hypothesized that twice-daily dosing has the potential to allow for more complete target engagement throughout the day at the level of the distal ileum. Intake of maralixibat 30 min prior to food consumption is based on the data of food effects study.

Weight-based dosing is proposed without a scientific rationale and requires justification for the age group, which was not evaluated in the clinical program (please refer to section 3.3.3).

Main study

LUM001-501

Methods

This was a completed phase 2 open-label exploratory long-term treatment study with maralixibat in the patients with PFIC1 and PFIC2. The study was initially designed as a 13-week study with a 59 week, long-term exposure period. Participants who completed the 59-week long-term exposure period to Week 72 had the option to continue in a long-term extension of the study (for > 5 years total duration), before rolling over into follow-up Study MRX-800.

Five protocol amendments were introduced during the study, affecting the inclusion/exclusion criteria, concomitant medication use and study medication.

Study Participants

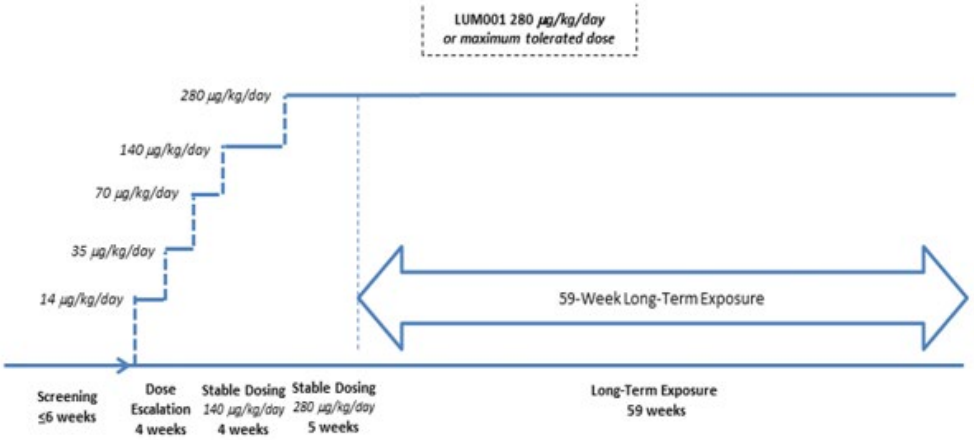
The study included patients with PFIC1 and PFIC2 from age of 12 months and up-to 18 years, inclusive. Genetic confirmation of the diagnosis (i.e. confirmation of a mutation at ATP8B1, or ABCB11) was not obligatory, provided that alternative causes of cholestasis were excluded. However, patients were offered genetic test during the study. Patients with elevated sBA (> 3xULN), low GGT levels,

without surgical disruption of the enterohepatic circulation or a liver transplant, concomitant decompensated cirrhosis, or any other relevant concomitant conditions were enrolled. There were no baseline pruritus eligibility criteria. The patients should not have been using bile acid or lipid binding resins, or sodium phenyl butyrate 30 days prior to study entry.

Treatments

Maralixibat was titrated slowly to the targeted therapeutic dose of 280 µg/kg/day (or maximum tolerated dose), which was maintained till end of the study (72 weeks in maintenance phase and during follow-up phase up-to over more than 5 years).

Figure 5 Study Design for LUM001-501 (Up to and Including Week 72) and titration schedule



The patients with insufficient response (elevated sBA and/or ItchRO ≥1.5 on 280 µg/kg/day QD maralixibat) were further escalated in the follow-up phase to the maximum of 560 µg/kg/day (280 µg/kg BID), by addition of a second (afternoon) dose of 280 µg/kg, which was titrated in 2 steps (2-weeks of 140 µg/kg, and 280 µg/kg).

During the long-term exposure period (Week 14 to end of trial (EOT)), the dose could be adjusted if there was a change of ≥10% in weight since screening visit. In case of intolerance, dose reduction was allowed. Maralixibat was taken at least 30 minutes prior to the first meal of the day and the second dose, where applicable, at least 30 minutes prior to dinner (evening meal). Participants were dosed orally using the dosing dispenser provided.

Concomitant drug therapy was to be kept stable, with the exception of weight-based dose adjustments and vitamin supplementation. No new medications to treat pruritus were allowed during the whole period of study till 2019 (introduction of the protocol amendment 4), when this restriction was limited to the first 13 weeks of treatment (reference period for primary endpoint). Bile acid or lipid binding resins and sodium phenylbutyrate were prohibited throughout the trial. The latter being forbidden via the protocol amendment 2.

Objectives

Overarching objective of the study was to evaluate safety and efficacy (short and long-term) of maralixibat in the patients with PFIC:

- To evaluate the long-term safety and tolerability of MRX in pediatric subjects with PFIC
- To evaluate the effect of MRX on serum bile acids in pediatric subjects with PFIC at 13 weeks of treatment

- To evaluate the effect of MRX on biochemical markers of cholestasis and liver disease at 13 weeks of treatment
- To evaluate the effect of MRX on pruritus in pediatric subjects with PFIC at 13 weeks of treatment

Outcomes/endpoints

The main focus for the analyses of efficacy was the period from Baseline (Day 0) to Week 13. For this period the following endpoints were defined for efficacy:

Primary efficacy endpoint:

- Fasting serum bile acid level change from Baseline (Day 0) to Week 13.

Secondary efficacy endpoints:

- Alanine aminotransferase (ALT) and bilirubin (total and direct) change from Baseline (Day 0) to Week 13.
- Pruritus as measured by ItchRO (Observer ItchRO/patient ItchRO) change from Baseline (Day 0) to Week 13. (For each subject, the average daily score was calculated using the 7 days pre-treatment for Baseline / Day 0, and the last 7 days of treatment for Week 13.)

Exploratory efficacy endpoints were evaluated over the period of 72 weeks:

- Change from Baseline (Day 0) in fasting serum bile acid level at Weeks 4, 8, 24, 36, 48 60, 72, 84, 96, 108, 120, every three months thereafter, and at the End of Treatment (EOT) visit.
- Change from Baseline (Day 0) in pruritus as measured by the average daily ItchRO (Observer ItchRO/patient ItchRO) at Weeks 4, 8, 28, 48, 86, 98, 110, 122, and every three months thereafter.
- Change from Baseline (Day 0) for ALT, and bilirubin (total and direct) at Weeks 4, 8, 24, 36, 48, 60, 72, 84, 96, 108, 120, every three months thereafter, and at the EOT visit.
- Responder analysis: pruritus response rates as measured by ItchRO (Observer ItchRO/patient ItchRO) at Weeks 4, 8, 13, 28, 48, 86, 98, 110, 122, and every three months thereafter, up to but not including the EOT visit.
- Change from Baseline (Day 0) in the Clinician Scratch Scale, at Weeks 2, 4, 8, 13, 24, 36, 48, 60, 72, 84, 96, 108, 120, every three months thereafter, and at the EOT visit.
- Change from Baseline (Day 0) for PedsQL at Weeks 13, 24, 48, 72, 84, 96, 108, 120, every three months thereafter, and at the EOT visit.

For the follow-up period (i.e. after week 72) the following additional endpoints were defined:

- Changes in height, weight, and BMI
- Change From Baseline for PedsQL Total Scale Score (Parent), Multidimensional Fatigue Scale Score (Parent), and Family Impact Total Scale Score.

Additionally to the above, Patient and Caregiver Impression of Change, Caregiver Global Therapeutic Benefit assessment, change from baseline for autotaxin and lysophosphatidic acid and responder analysis based on various definitions of response (change in sBA, AST, ALT, bilirubin, C4, pruritus) were assessed.

A post hoc subgroup efficacy analysis of responder vs. non-responder patients, subgroup analysis of patients with nt-PFIC (non-truncated) and t-PFIC2 (truncated) subtypes and transplant-free survival analysis (TFS) in sBA responder (sBA < 102 µmol/L, or ≥ 75% reduction from baseline; van Wessel et al., 2020) vs non-responder patients was conducted.

Randomisation and blinding (masking)

This was an open-label single arm study. No randomisation was performed and blinding was N/A.

Statistical methods

The Week 72 analysis, which was the 3rd interim analysis, was the subject of the final CSR. A final analysis was performed after the last participant transitioned to the rollover Study MRX-800 and the Study LUM001-501 database was locked. The results of the interim analyses may have led to changes in the study design. Staff directly involved in the conduct of the study reviewed the results of the interim analyses.

The mITT Population, being defined as all subjects who were assigned, received at least 1 dose of treatment, and have at least 1 post-baseline primary efficacy assessment, was the main population for efficacy analysis for the 3rd planned interim analysis. The data for only 1 sibling was to be considered for the mITT and PP populations. For the final analysis, an intent-to-treat (ITT) analysis population including each sibling pair has been added as main analysis population.

Data summaries are presented for PFIC1 and PFIC2 subjects independently and for all subjects combined.

The main focus for the analyses of efficacy was the period from Baseline (Day 0) to Week 13. The primary analysis of the primary efficacy endpoint change from baseline in fasting sBA was based on a paired t-test. The null hypothesis that the mean change from baseline to Week 13/ET is zero was tested. Secondary efficacy variables were analyzed similar to the primary efficacy analyses. No adjustment for multiplicity were made.

Efficacy endpoints analyzed by time point were defined as the last post-baseline value obtained within 7 days of the date of "last dose" within the defined study period, i.e. last observation carried forward was applied for missing data.

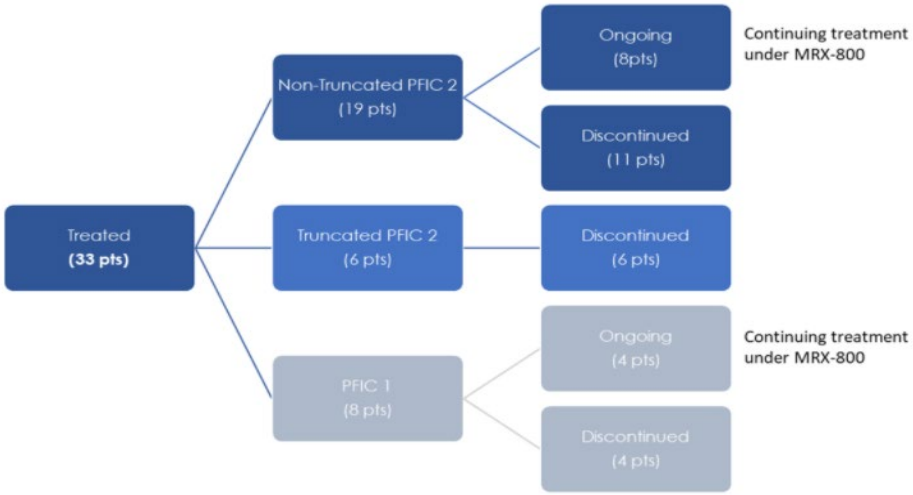
Results

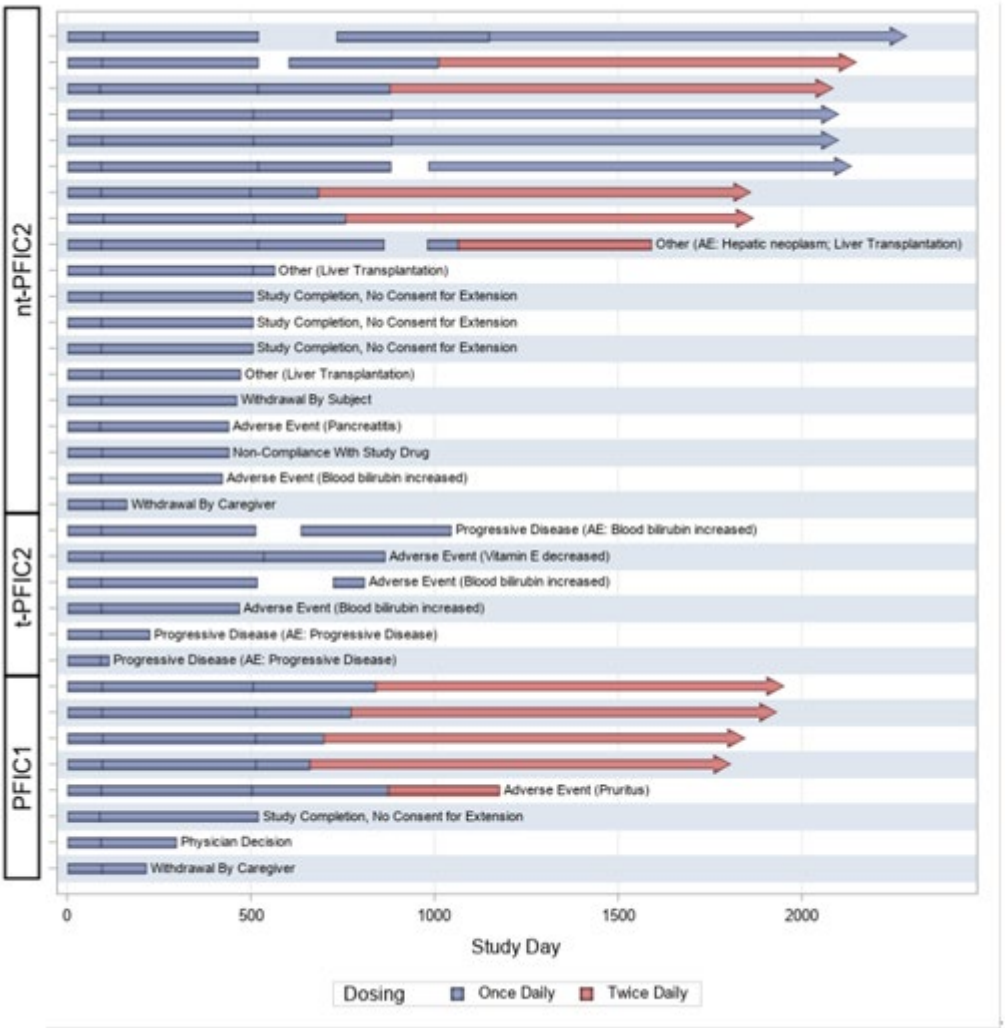
The study was conducted at 11 sites in 4 countries (United States, United Kingdom, France, and Poland). The study centres were mainly hospital-based paediatric centres specialised in liver diseases. First participant was screened on 12 February 2014 and last subject last visit took place on 20 May 2020.

As the targeted indication is PFIC2, the primary focus in the analysis and data presentation is put on the PFIC2 subpopulation of the study.

Participant flow

Figure 6 Study Participant flow





In total 37 patients were screened and 33 patients with PFIC (8 with PFIC1 and 25 with PFIC2, including 19 nt-PFIC2 and 6 t PFIC2) were enrolled in the study. Majority (68%) of the population with

PFIC2 (17/25 patients) discontinued the study. Predominant reasons of discontinuation were refusal to continue study treatment/participation (i.e. non-compliance/stopping treatment, withdrawal of consent either by patient or caregiver, refusal to continue treatment in follow-up study; 6/25 [24%]) and disease progression/liver transplantation (6/25 [24%] of patients with PFIC2; 3/19 of patients with nt-PFIC2 and 3/6 patients with t-PFIC2). Remaining patients with PFIC2 (5/25; 20%) withdrew due to AEs.

Baseline data

Baseline demographic information and disease characteristics for the study population are presented in the tables below.

Table 9 Demographics and Baseline Characteristics (Safety Population; Overall)

Variable Statistic or Category	PFIC1 (N=8)	PFIC2 nt-PFIC2 (N=19)	PFIC2 t-PFIC2 (N=6)	PFIC2 PFIC2 Overall (N=25)	Overall (N=33)
Age, in years[a]					
Mean (SE)	3.0 (0.71)	4.1 (0.79)	6.3 (1.38)	4.6 (0.70)	4.2 (0.56)
Median	2.0	3.0	7.0	4.0	3.0
Min, Max	1, 7	1, 13	1, 10	1, 13	1, 13
Age category,[a] n (%)					
< 2 years	1 (12.5)	5 (26.3)	1 (16.7)	6 (24.0)	7 (21.2)
2 to 4 years	5 (62.5)	9 (47.4)	1 (16.7)	10 (40.0)	15 (45.5)
5 to 8 years	2 (25.0)	2 (10.5)	2 (33.3)	4 (16.0)	6 (18.2)
9 to 12 years	0	2 (10.5)	2 (33.3)	4 (16.0)	4 (12.1)
13 to 18 years	0	1 (5.3)	0	1 (4.0)	1 (3.0)
Gender, n (%)					
Male	6 (75.0)	6 (31.6)	2 (33.3)	8 (32.0)	14 (42.4)
Female	2 (25.0)	13 (68.4)	4 (66.7)	17 (68.0)	19 (57.6)
Height z-score					
Mean (SE)	-2.963 (0.5194)	-1.066 (0.2296)	-1.982 (0.2144)	-1.286 (0.1968)	-1.693 (0.2290)
Weight z-score					
Mean (SE)	-2.704 (0.9963)	-0.402 (0.1764)	-1.331 (0.3607)	-0.625 (0.1755)	-1.129 (0.3081)
Race, n (%)					

Variable Statistic or Category	PFIC1 (N=8)	PFIC2 nt-PFIC2 (N=19)	PFIC2 t-PFIC2 (N=6)	PFIC2 PFIC2 Overall (N=25)	Overall (N=33)
American Indian or Alaska Native	0	0	0	0	0
Asian	2 (25.0)	0	1 (16.7)	1 (4.0)	3 (9.1)
Black or African American	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
White	6 (75.0)	18 (94.7)	2 (33.3)	20 (80.0)	26 (78.8)
More than 1 race	0	1 (5.3)	0	1 (4.0)	1 (3.0)
Not reported	0	0	3 (50.0)	3 (12.0)	3 (9.1)
Ethnicity, n (%)					
Hispanic or Latino	0	1 (5.3)	0	1 (4.0)	1 (3.0)
Not Hispanic or Latino	8 (100.0)	18 (94.7)	3 (50.0)	21 (84.0)	29 (87.9)
Not Reported	0	0	3 (50.0)	3 (12.0)	3 (9.1)
Country, n (%)					
France	0	0	3 (50.0)	3 (12.0)	3 (9.1)
Great Britain	3 (37.5)	9 (47.4)	2 (33.3)	11 (44.0)	14 (42.4)
Poland	0	1 (5.3)	0	1 (4.0)	1 (3.0)
United States	5 (62.5)	9 (47.4)	1 (16.7)	10 (40.0)	15 (45.5)
sBA, in µmol/L					
Mean (SE)	261.81 (35.205)	373.35 (37.154)	404.92 (45.886)	380.93 (29.995)	352.05 (25.658)
ALT, in U/L					
Mean (SE)	56.1 (10.51)	116.0 (25.04)	152.3 (61.63)	124.7 (23.61)	108.1 (18.70)
AST, in U/L					
Mean (SE)	77.3 (8.40)	147.9 (37.88)	205.7 (68.30)	161.8 (32.81)	141.3 (25.62)
GGT, in U/L					
Mean (SE)	17.8 (2.14)	21.9 (4.37)	50.2 (10.67)	28.7 (4.76)	26.1 (3.72)

Variable Statistic or Category	PFIC1 (N=8)	PFIC2 nt-PFIC2 (N=19)	PFIC2 t-PFIC2 (N=6)	PFIC2 PFIC2 Overall (N=25)	Overall (N=33)
Bilirubin, in mg/dL					
Mean (SE)	5.45 (1.814)	1.81 (0.409)	2.87 (0.610)	2.06 (0.350)	2.88 (0.557)
ItchRO(Obs) Weekly Morning Average Score					
Mean (SE)	2.307 (0.3045)	2.090 (0.1890)	2.929 (0.2045)	2.291 (0.1667)	2.295 (0.1439)
Clinician Scratch Scale Score					
Mean (SE)	2.8 (0.16)	2.9 (0.24)	2.7 (0.21)	2.9 (0.19)	2.8 (0.15)

GGT = gamma glutamyltransferase; ItchRO(Obs) = Itch Reported Outcome – Observer; Max = maximum; Min = minimum; n = number in a given category; N = number of participants; nt-PFIC2 = non-truncating PFIC2; PFIC = progressive familial intrahepatic cholestasis; sBA = serum bile acid; SE = standard error of the mean; t-PFIC2 = truncating PFIC2.

Note: Percentages are 100*n/N.

Age at time of baseline visit.

Source: LUM001-501 Final CSR Addendum, Table 14.1.3, Table 14.1.4, Table 14.2.3.2.1, Table 14.2.3.2.2.

Table 10 Disease History and Baseline Disease Characteristics (Safety Population: Overall)

Variable Statistic or Category	PFIC1 (N=8)	PFIC2 nt-PFIC2 (N=19)	PFIC2 t-PFIC2 (N=6)	PFIC2 PFIC2 Overall (N=25)	Overall (N=33)
Time Since Original Diagnosis of PFIC, in months					
Median (minimum, maximum)	23.38 (12.5, 78.9)	27.14 (4.6, 118.6)	54.88 (18.5, 127.0)	33.08 (4.6, 127.0)	32.23 (4.6, 127.0)
Family History of PFIC, n (%)					
Yes	3 (37.5)	3 (15.8)	0	3 (12.0)	6 (18.2)
No	5 (62.5)	15 (78.9)	3 (50.0)	18 (72.0)	23 (69.7)
Unknown	0	1 (5.3)	3 (50.0)	4 (16.0)	4 (12.1)
Used Anything to Treat Itch in the Past, n (%)					

		PFIC2			
Variable Statistic or Category	**PFIC1 (N=8)**	**nt-PFIC2 (N=19)**	**t-PFIC2 (N=6)**	**PFIC2 Overall (N=25)**	**Overall (N=33)**
Yes	7 (87.5)	16 (84.2)	5 (83.3)	21 (84.0)	28 (84.8)
No	1 (12.5)	3 (15.8)	1 (16.7)	4 (16.0)	5 (15.2)
Type of Therapy Used to Treat Itch in the Past,[a] n (%)					
Topical	1 (12.5)	3 (15.8)	1 (16.7)	4 (16.0)	5 (15.2)
Oral	7 (87.5)	15 (78.9)	5 (83.3)	20 (80.0)	27 (81.8)
Other	2 (25.0)	9 (47.4)	0	9 (36.0)	11 (33.3)
Specific Therapy Used to Treat Itch in the Past, n (%), Most Common (> 20% Overall)					
Oral Enzyme Inducers	5 (62.5)	12 (63.2)	5 (83.3)	17 (68.0)	22 (66.7)
Oral Ursodiol	5 (62.5)	14 (73.7)	3 (50.0)	17 (68.0)	22 (66.7)
Oral Antihistamines	3 (37.5)	11 (57.9)	1 (16.7)	12 (48.0)	15 (45.5)
Oral Binding Resins	2 (25.0)	9 (47.4)	1 (16.7)	10 (40.0)	12 (36.4)
Other	2 (25.0)	7 (36.8)	0	7 (28.0)	9 (27.3)
Oral Opiate Antagonists	2 (25.0)	5 (26.3)	1 (16.7)	6 (24.0)	8 (24.2)
Oral Serotonin Antagonists	2 (25.0)	5 (26.3)	0	5 (20.0)	7 (21.2)

n = number in a given category; N = number of participants; nt-PFIC2 = non-truncating PFIC2; PFIC = progressive familial intrahepatic cholestasis; SE = standard error of the mean; t-PFIC2 = truncating PFIC2.

Note: Percentages are 100*n/N.

[a] Each medication/therapy was prespecified on the case report form; see LUM001-501 Final CSR Addendum, Table 14.1.5 for complete list.

Source: LUM001-501 Final CSR Addendum, Table 14.1.4.

Genotype analysis

Results of genotype analysis of only 11 out of 19 patients with nt-PFIC2 are listed in the documentation (listing 16.12; appendix 8.2.9) and data of only 2 out of 6 patients with t-PFIC2. Mutations in gene ABCB11 were confirmed in 10 patients with nt-PFIC2 and both patients with t-PFIC2. One patient (0016054) had additional "other" mutation and in one patient (0016052) no mutation on ABCB11 was detected.

Numbers analysed

Safety and ITT data sets for PFIC2 population include 25 patients (19 with nt-PFIC2 and 6 with t-PFIC2). Additionally, for the efficacy analysis an mITT set was defined, which included 23 patients with PFIC2. PP analysis was planned but could not be located.

Outcomes and estimation

Primary endpoint

The primary efficacy endpoint in PFIC population, change from baseline to Week 13/ET in fasting sBA level, was not met. No relevant changes to baseline were observed in either patient subgroup for mean and median values. Mean (SD; median; Min, Max) numerical reduction in sBA by 38 µmol/L (177.7; -11; - 463; 279) was observed at Week 13/ET in the mITT population from the baseline mean (SD) value of 393 (144.6) µmol/L.

Secondary endpoints

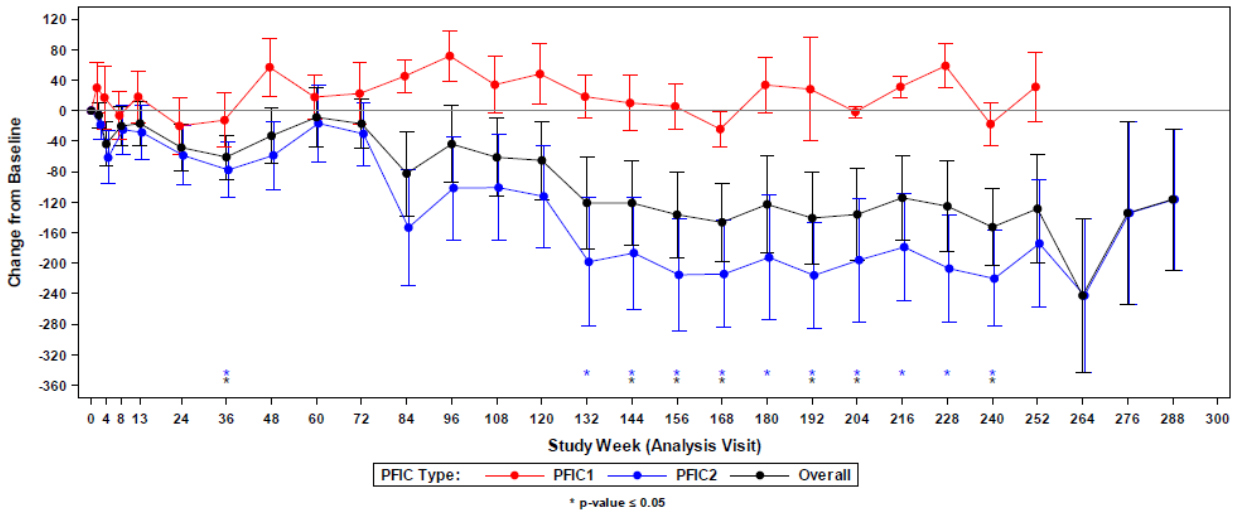
At week 13 ALT, total bilirubin, and direct bilirubin showed no relevant changes from baseline.

Numerical and statistically significant improvement was observed in the PFIC2 population in mean (SD) ItchRO(Obs) 4-week average morning score (-0.715 (0.1179) (95% CI for Mean Difference from Baseline: -0.959, -0.472; p<0.0001)) at week 13/EOT, that did not reach clinically meaningful threshold of >1 point, as defined by the applicant.

Exploratory endpoints and efficacy data on long-term treatment

Numerical reduction in mean sBA levels from baseline was observed in the participants with PFIC2. These reductions were more pronounced in participants with nt-PFIC2, than those with t-PFIC2; statistically significant reductions from baseline were observed at Weeks 2, 4, 36, 108, and 120 through 240 in participants with nt-PFIC2, whereas no statistically significant reductions from baseline were observed for participants with t-PFIC2.

Figure 7 Mean (SE) Change From Baseline in Serum Bile Acid Level (µmol/L) by PFIC Type (Intent-to-Treat Population)

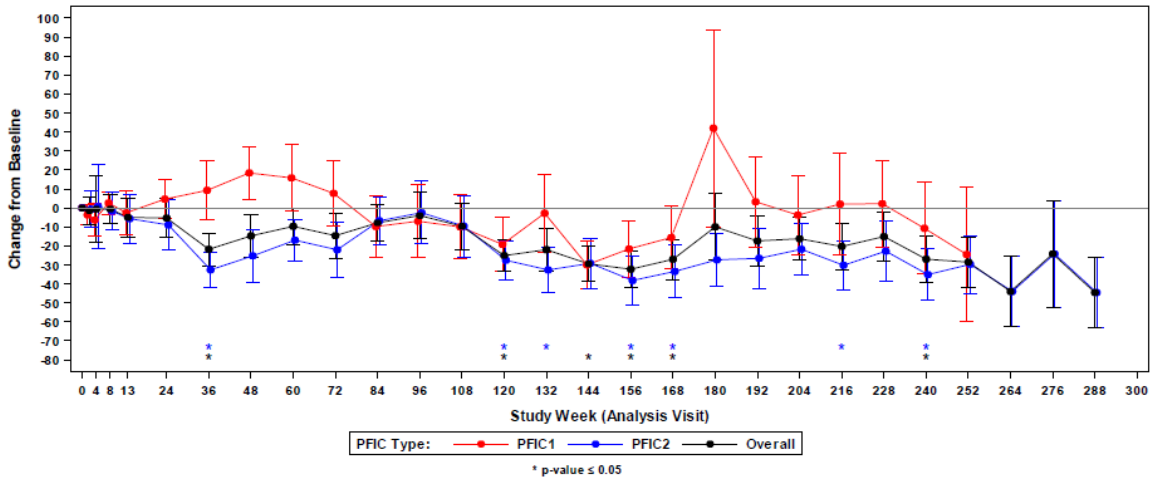


Source: Appendix 8.1, Figure 14.2.2.1.1
Abbreviations: CFB = change from baseline; PFIC = progressive familial intrahepatic cholestasis; SE = standard error of the mean
Note: Baseline was defined as the last observation obtained before the first dose. Only analysis visits with an overall n ≥4 were summarized. Participants that did not complete at least 1 post-Week 72 assessment were excluded from all post-Week 72 analyses. Participants that did not complete at least 1 post-Week 124 assessment were excluded from all post-Week 124 analyses. * Paired t-test used to test if mean change is statistically significant (null hypothesis is that the mean CFB is equal to zero).

Changes from baseline in the levels of ALT, AST and total bilirubin are presented in the figures below.

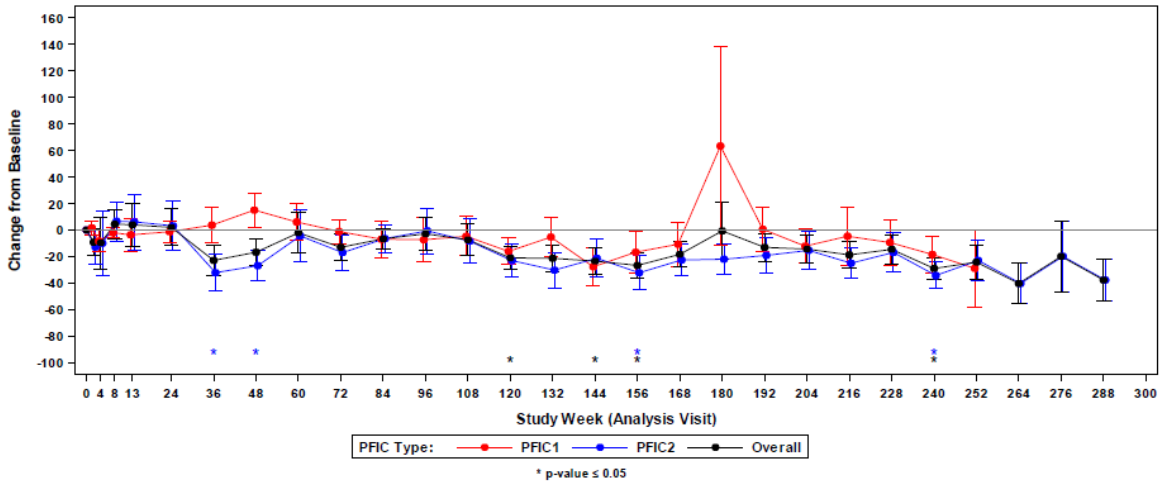
Figure 8 Change to baseline ALT, AST and total Bilirubin in PFIC, PFIC1 and PFIC2 patient populations (a), b) and c) in ITT)

a) ALT (U/L)



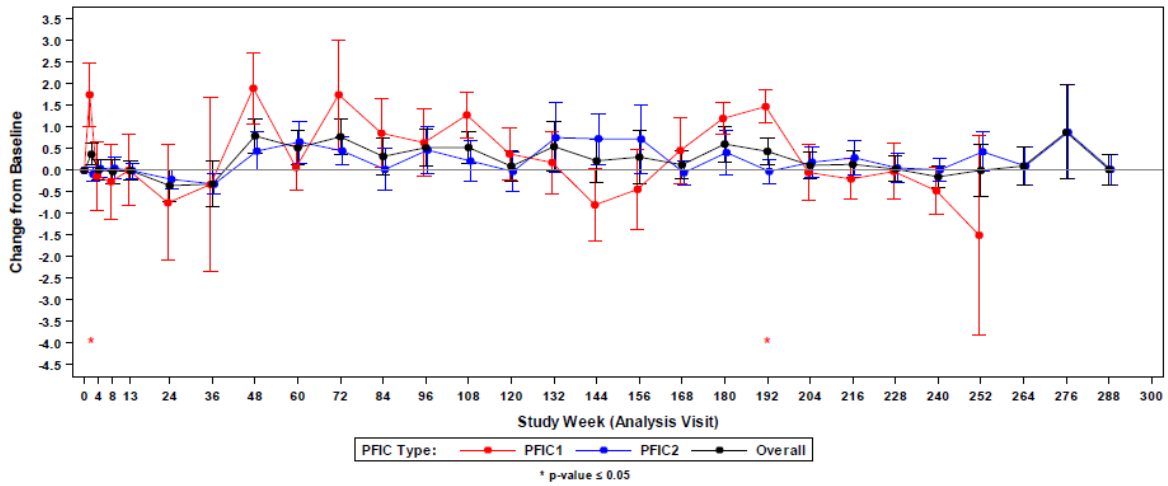
Source: Appendix 8.1, Figure 14.2.5.1
Abbreviations: CFB = change from baseline; PFIC = progressive familial intrahepatic cholestasis; SE = standard error of the mean
Note: Baseline was defined as the last observation obtained before the first dose. Only analysis visits with an overall n ≥4 were summarized. Participants that did not complete at least 1 post-Week 72 assessment were excluded from all post-Week 72 analyses. Participants that did not complete at least 1 post-Week 124 assessment were excluded from all post-Week 124 analyses. * Paired t-test used to test if mean change is statistically significant (null hypothesis is that the mean CFB is equal to zero).

b) AST (U/L)



Source: Appendix 8.1, Figure 14.2.9.1
Abbreviations: CFB = change from baseline; PFIC = progressive familial intrahepatic cholestasis; SE = standard error of the mean
Note: Baseline was defined as the last observation obtained before the first dose. Only analysis visits with an overall n ≥4 were summarized. Participants that did not complete at least 1 post-Week 72 assessment were excluded from all post-Week 72 analyses. Participants that did not complete at least 1 post-Week 124 assessment were excluded from all post-Week 124 analyses. * Paired t-test used to test if mean change is statistically significant (null hypothesis is that the mean CFB is equal to zero).

c) Total bilirubin (mg/dL)



Source: Appendix 8.1, Figure 14.2.6.1
Abbreviations: CFB = change from baseline; PFIC = progressive familial intrahepatic cholestasis; SE = standard error of the mean
Note: Baseline was defined as the last observation obtained before the first dose. Only analysis visits with an overall n ≥4 were summarized. Participants that di not complete at least 1 post-Week 72 assessment were excluded from all post-Week 72 analyses. Participants that did not complete at least 1 post-Week 12 assessment were excluded from all post-Week 124 analyses. * Paired t-test used to test if mean change is statistically significant (null hypothesis is that the mean CFB is equal to zero).

Statistically significant reductions in mean values of ItchRO(Obs) weekly morning average score was observed at all time points with the exception of Week 134 (which included data for only 3 participants). Improvements in pruritus were less prominent in the 6 patients with t-PFIC2 subtype with maximum of change of – 1.357 (p>0.05) reached at week 110.

In addition, the overall cohort (PFIC1 and 2) experienced a > 1.0-point reduction (the threshold for clinical meaningfulness) from Week 48 onward.

Table 11 Change from Baseline in ItchRO (Observer) Weekly Morning Average Score by PFIC2 Subtype

Visit	PFIC2		nt-PFIC2	
Statistic	**Observed**	**CFB**	**Observed**	**CFB**
Baseline				
n	25		19	
Mean (SE)	2.291 (0.1667)		2.090 (0.1890)	
Week 13				
n	25	25	19	19
Mean (SE)	1.542 (0.1832)	-0.750 (0.1477)	1.337 (0.1891)	-0.753 (0.1800)
p-value for CFB[a]		< 0.0001		0.0006
Week 48				
n	22	22	18	18
Mean (SE)	1.149 (0.1952)	-1.058 (0.1858)	1.062 (0.2062)	-0.978 (0.2095)
p-value for CFB[a]		< 0.0001		0.0002
Week 146				

n	10	10	9	9
Mean (SE)	0.700 (0.2788)	-1.671 (0.3368)	0.556 (0.2667)	-1.746 (0.3671)
p-value for CFB[a]		0.0008		0.0014
Week 254				
n	6	6	6	6
Mean (SE)	0.220 (0.1412)	-1.970 (0.2925)	0.220 (0.1412)	-1.970 (0.2925)
p-value for CFB[a]		0.0011		0.0011

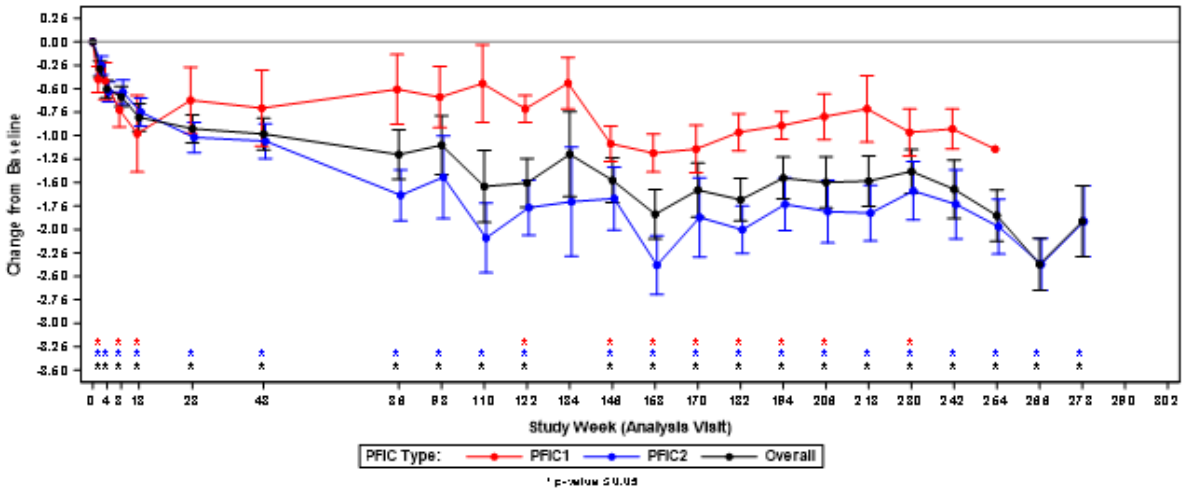
CFB = change from baseline, defined as postbaseline value minus baseline value; n = number in a given category; PFIC = progressive familial intrahepatic cholestasis; SE = standard error.

Note: ItchRO scores have a range from 0 to 4, with the higher score indicating increasing itch severity.

[b] P-value for testing if the mean change from baseline at the specified analysis visit is zero, using a paired t-test.

Source: LUM001-501 Final CSR Addendum, Table 14.2.3.2.2.

Figure 9 Change from Baseline in ItchRO (Observer) Weekly Morning Average Score by PFIC Type Over Time (Intent-to-Treat Population)



a.

ItchRO(Obs) = Itch Reported Outcome (Observer); PFIC = progressive familial intrahepatic cholestasis; SE = standard error of the mean

Note: Baseline was defined as the average of daily scores in the week consisting of the 7 days immediately before the first dose. Only analysis visits with an overall n ≥4 were summarized. Participants that did not complete at least 1 post-Week 72 assessment were excluded from all post-Week 72 analyses. Participants that did not complete at least 1 post-Week 124 assessment were excluded from all post-Week 124 analyses. * Paired t-test used to test if mean change is statistically significant (null hypothesis is that the mean CFB is equal to zero).

Source: LUM001-501 Final CSR Addendum, Appendix 8.1, Figure 14.2.3.2.1.1.

ItchRO(Obs) average weekly and average evening weekly scores, ItchRO(Pt) (n=9) and clinician's scratch scores (CSS) showed the results consistent to those of ItchRO(Obs) morning scores.

No relevant changes were observed for z-scores for height, weight or BMI in the patients with PFIC2. Worsening of z-score for height was observed in the PFIC1 subgroup of patients.

Mean and median scores of PedsQL Total Scale Score (Parent) and Multidimensional Fatigue Scale Score (Parent) showed improvements in PFIC2 population compared to baseline in the follow-up phase of the study. No changes were reported in Family Impact Total Scale Score. Impact on sleep was not discussed.

Results of Patient Impression of Change (PIC), Caregiver Impression of Change (CIC), Caregiver Global Therapeutic Benefit (CGTB) were not summarized.

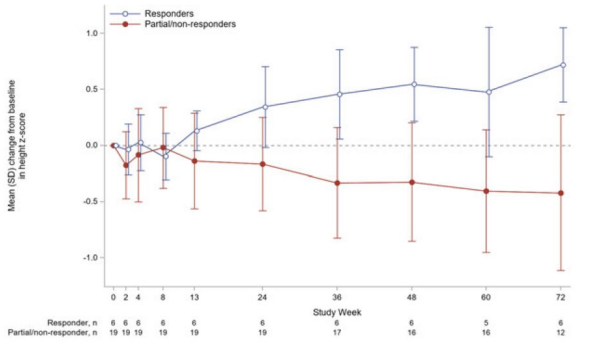
Analyses of cholestasis biomarkers were not discussed in the dossier. Unconjugated bile acids increased, conjugated decreased (especially after week 72 of treatment), and mean values of C4 increased during treatment with maralixibat in the patients with PFIC2. Autotaxin levels seemed to decrease, but frequent fluctuations in visit-to-visit values were observed. Mean values of FGF19 levels fluctuated over the whole treatment period, without clear trends in change. However, median values indicated reduction at all visits compared to baseline.

Changes in the concomitant medication were analysed for rifampicin and UDCA only and showed that dosing regimen was changed in 19/25 patients (including increased dosing in 4 and decrease or cessation of use in 11 subjects). Use of additional antipruritic medications and other concomitant medications was noted in the study, but no data are available.

Responder analyses

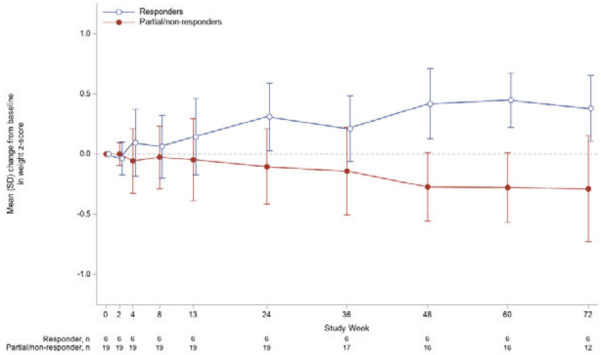
Responder analysis (i.e. "multiparameter responders") based on 2 criteria a) a 70% reduction or normalization of sBA and b) reduction of 1.0 point in ItchRO(Obs) weekly morning average, showed that 6 participants with PFIC2 out of 25 met the response criteria across multiple timepoints from Week 4 to Week 72 and exhibited reductions in ALT, AST, and bilirubin in cases where those values were elevated at baseline. Height and weight z-score changes from baseline in participants who responded to treatment differed in a clinically significant fashion from non-responders.

Figure 10 z-scores for height (A) and weight (B) in subgroups of responders (blue line) vs. non-responders/partial responders (red line)(ITT)



Source: Figure provided by Mirum Pharmaceuticals, Inc., using data from Appendix 8.2, Listing 16.2.7.7 (see Appendix 8.1.4, Figure 14.4.1)
Abbreviation: n = number in a given category; SD = standard deviation
Note: mean (SD) data values can be provided by Mirum Pharmaceuticals, Inc. upon request.

(A)



Source: Figure provided by Mirum Pharmaceuticals, Inc., using data from Appendix 8.2, Listing 16.2.7.7 (see Appendix 8.1.4, Figure 14.4.2)
Abbreviation: n = number in a given category; SD = standard deviation
Note: mean (SD) data values can be provided by Mirum Pharmaceuticals, Inc. upon request.

(B)

Analysis of the baseline characteristics of the responder vs non/partial responders showed no major differences in age, sex, height, weight, or race. All responders had nt-PFIC2 mutation (i.e., all t-PFIC2 were non-responders) and significantly lower (or even normal in case of bilirubin) levels of ALT, AST, and total bilirubin when compared to non-responders (i.e. patients including nt-PFIC2 and t-PFIC2).

The mean baseline levels of sBA in responders were roughly in line with those reported in the literature (van Wessel et al., 2020), but levels of ALT, AST, and total bilirubin, are not consistent with/clearly below those reported in the literature (van Wessel et al., 2020).

High proportion of the responders was reported in the US (5/10), with 4 out 5 responder patients enrolled in one site.

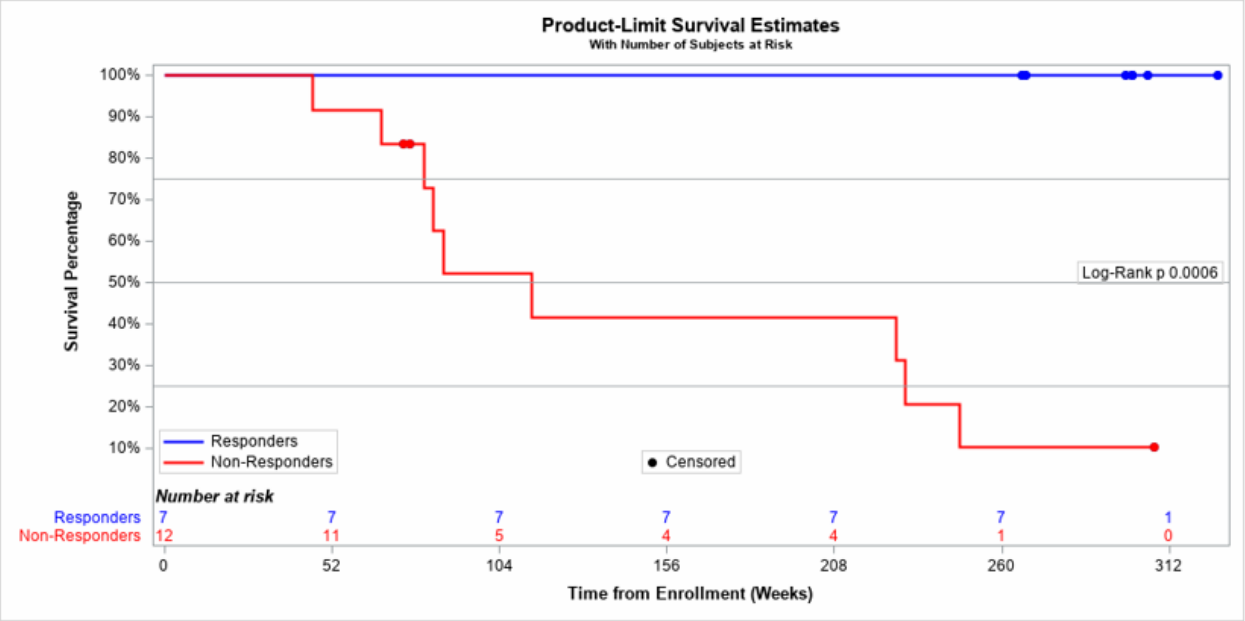
Ancillary analyses

A retrospective analysis of long-term clinical outcome effects on transplant free survival (TFS) between sBA responders and nonresponders with PFIC2, treated with maralixibat in Study LUM001-501

An additional responder analysis on TFS was conducted on the long-term LUM001-501 nt-PFIC2 study participants. In order to provide a complete dataset, Mirum collected follow-up information on clinical events on all nt-PFIC2 participants who discontinued from Study LUM001-501. An analysis was conducted on maralixibat sBA response compared with maralixibat sBA nonresponse, with response defined as sBA <102 µmol/L or 75% reduction compared with baseline (hereafter referred to as sBA responders). Time to occurrence of liver transplant in maralixibat responders compared with nonresponders was performed.

Results from this analysis demonstrated that 100% of study participants who achieved the sBA response criteria remain transplant free, whereas the majority of participants without sBA response went on to receive a liver transplant.

Figure 11 Transplant-free survival – responder vs. non-responders in nt-PFIC2 subgroup



nt PFIC2 = nontruncating progressive familial intrahepatic cholestasis 2; sBA = serum bile acid. Data Cutoff Date: 30 July 2020.

Responder is defined as control of sBA to < 102 µm/L or a 75% reduction compared with baseline within 1 year after highest dose administered.

Source: LUM001-501 vs NAPPED Report, Figure 2.

The analysis was repeated to include the 6 maralixibat-treated t-PFIC2 participants from Study LUM001-501, for a total of 25 participants (19 nt-PFIC2 and 6 t-PFIC2). Results from this combined nt-

PFIC2 and t-PFIC2 groups were shown to be comparable to the nt-PFIC2 analysis and were also statistically significant (p=0.0018).

Summary of main efficacy results

The following table summarises the efficacy results from the main study supporting the present application. Focus is put on the population targeted (PFIC2). This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 12 Summary of efficacy for trial LUM001-501

Title: INDIGO STUDY: Open Label Study of the Efficacy and Long Term Safety of LUM001, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi), in the Treatment of Cholestatic Liver Disease in Pediatric Patients with Progressive Familial Intrahepatic Cholestasis			
Study identifier	LUM001-501 EuDRA CT No: 2013-003833-14		
Design	An open-label single-arm multi-centre phase 2 study with follow-up treatment period		
	Duration of main phase: Run-in phase: Duration of Extension phase:	13 weeks (primary endpoint)/72 weeks N/A up-to more than 5 years	
Hypothesis	Superiority: Change from baseline		
Treatments groups	All patients received maralixibat starting with 14 µg/kg/day QD (1 week), followed with 35 µg/kg/day QD (1 week), 70 µg/kg/day QD (1 week), 140 µg/kg/day QD (5 weeks), and 280 µg/kg/day QD (therapeutic dose) till end of study. Titration was stopped in case of intolerance. Patients in follow-up phase with insufficient response were escalated to 280 µg/kg BID (560 µg/kg/day dose). Down-titration was allowed at any time.		
Endpoints and definitions	Primary endpoint	CFB in sBA at Week 13/ET	Change from baseline to Week 13/ET in fasting sBA level in patients with PFIC
	Secondary endpoints	CFB in ALT at Week 13/ET	Change from baseline to Week 13/ET in mean ALT
	Secondary endpoints	CFB in total bilirubin at Week 13/ET	Change from baseline to Week 13/ET in mean total bilirubin
	Secondary endpoints	CFB in direct bilirubin at Week 13/ET	Change from baseline to Week 13/ET in mean direct bilirubin
	Secondary endpoints	CFB in ItchRO (Obs) 4-Weeks average morning score 13/ET	Change from baseline to Week 13/ET ItchRO(Obs) 4-weeks average morning score
	Secondary endpoints	CFB in ItchRO(Pt) 4-Weeks average morning score 13/ET	Change from baseline to Week 13/ET ItchRO(Pt) 4-weeks average morning score
	Exploratory long-term treatment	Multiparameter responder analyses	Responder defined as at least 1 point decrease in average weekly ItchRO(Obs) score from baseline and sBA < 102 µm/L or a 75% reduction compared with baseline
	Post hoc long-term treatment	z-scores for height and weight in responder	Development of z-score for height and weight compared to baseline and between "sBA responders" and non-responders (responder defined as sBA < 102 µm/L or a 75% reduction compared with baseline)
	Post hoc	TFS in responder vs. non-responder	TFS analysis between "sBA responders" and non-responders (responder defined as sBA < 102 µm/L or a 75% reduction compared with baseline)

Database lock	20 Jul 2020 (Last Participant Last Visit: 08 May 2020)		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Modified ITT Week 13/ET		
Descriptive statistics and estimate variability	Treatment group	PFIC2	PFIC
	Number of subjects	23	31
	CFB in sBA at Week 13/ET (mean)	-38	-23
	95% CI	-114.598, 39.082	-82.350, 35.742
	SD	177.7	161
Effect estimate per comparison	Not applicable.		
Notes	The study had only one treatment arm. Comparisons were made to the baseline. Primary analysis was conducted for PFIC population, that does not reflect the targeted population.		
Analysis description	**Secondary endpoints (pre-specified) - 1**		
Analysis population and time point description	Modified ITT Week 13/ET		
Descriptive statistics and estimate variability	Treatment group	PFIC2	PFIC
	Number of subjects	23	31
	CFB in ALT at Week 13/ET (mean)	-11.3	-9
	95% CI	-41.6, 19.0	- 31.6, 13.7
	SD	70.1	61.8
Analysis description	**Secondary endpoints (pre-specified) - 2**		
Analysis population and time point description	Modified ITT Week 13/ET		
Descriptive statistics and estimate variability	Treatment group	PFIC2	PFIC
	Number of subjects	23	31
	CFB in total bilirubin at Week 13/ET (mean)	-0.02	-0.21
	95% CI	-0.41, 0.37	-0.82, 0.40
	SD	0.9	1.7
Analysis description	**Secondary endpoints (pre-specified) - 3**		
Analysis population and time point description	Modified ITT Week 13/ET		
Descriptive statistics	Treatment group	PFIC2	PFIC

and estimate variability	Number of subjects	23	31
	CFB in direct bilirubin at Week 13/ET (mean)	-0.026	-0.094
	95% CI	-0.335, 0.283	-0.505, 0.318
	SD	0.7	1.1
Analysis description	**Secondary endpoints (pre-specified) - 4**		
Analysis population and time point description	Modified ITT Week 13/ET		
Descriptive statistics and estimate variability	Treatment group	PFIC2	PFIC
	Number of subjects	23	31
	CFB in ItchRO(Obs) 4-Week average morning score at 13/ET (mean)	-0.697	-0.731
	95% CI	-0.954, -0.441	-0.969, -0.492
	SD	0.6	0.7
Analysis description	**Secondary endpoints (pre-specified) - 6**		
Analysis population and time point description	Modified ITT Week 13/ET		
Descriptive statistics and estimate variability	Treatment group	PFIC2	PFIC
	Number of subjects	7	9
	CFB in ItchRO(Pt) 4-Week average morning score at 13/ET (mean)	-0.705	-0.633
	95% CI	-1.234, -0.175	-1.067, -0.198
	SD	0.6	0.6
Analysis description	**Secondary endpoints (post hoc) - 7**		
Analysis population and time point description	Modified ITT Long-term treatment		
Descriptive statistics and estimate variability	Treatment group	PFIC2	
	Number of subjects	25	
	Multiparameter responders	6/25 (24%)	
Note	None of the patients with t-PFIC2was responder		
Analysis description	**Secondary endpoints (post hoc) - 8**		
Analysis population and time point description	Modified ITT Long-term treatment		
Descriptive statistics and estimate variability	Treatment group	PFIC2	
	Number of subjects	25	
	z-Scores for height and weight	Mean values of z-scores for height and weight improved in responder, but not in non-responders	
Analysis description	**Secondary endpoints (post hoc) - 9**		

Analysis population and time point description	Modified ITT Long-term treatment	
Descriptive statistics and estimate variability	Treatment group	PFIC2
	Number of subjects	25
	TFS in sBA responders	TFS amounted to 100% in responders (p=0.0018)

Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable.

Clinical studies in special populations

Not applicable.

Supportive study(ies)

LUM001-501 vs. NAPPED (historical data)

Study title: Outcomes of Long-Term Maralixibat Treatment in Participants with PFIC2; Comparison of LUM001-501 with External Natural History Controls from the NAPPED Control Group.

Ongoing studies

There are 3 ongoing studies with maralixibat in participants with PFIC: Studies MRX-800, MRX 502, and MRX-503.

Study MRX 800 is a long-term extension study to evaluate the long-term safety of maralixibat for participants with PFIC who have completed Study LUM001-501, with secondary objectives to evaluate the long-term effect of maralixibat on pruritus, sBA levels, time to liver-associated events, and growth.

Study MRX-502 is a 26-week, double-blind, placebo-controlled study with a primary objective to evaluate the efficacy and safety of higher doses (up to 600 µg/kg twice daily) of maralixibat on short-term endpoints. The primary endpoint is the severity of pruritus. The planned enrollment targets 30 participants with nt-PFIC2 and up to 60 participants with other PFIC subtypes.

Study MRX-503 is a 104-week, long-term, open label extension to Study MRX-502. Maintenance of the sBA and pruritus treatment effect over 2–2.5 years will be assessed, as well as mid- to long term benefits on growth and EFS.

The program submitted to support this application is strictly limited in terms of size and quality of the database. Key evidence of efficacy is provided from one small Phase 2 exploratory, non-randomised, single-arm study and is limited to 25 patients with PFIC2 (LUM001-501; pivotal study) in the age range of 1 to 13 years, including. Additional supportive data are derived from a retrospective comparison of the data from the pivotal study and of the patients from the natural history database (NAPPED registry) as an external control. Analysis was done on event free survival (EFS) (events defined as liver transplantation, surgery of biliary diversion (SBD), or death). No data have been provided from the ongoing studies in PFIC population. Although, clinical studies with maralixibat in Alagille syndrome (another condition characterised with cholestasis) were conducted, due to the

different pathophysiology efficacy data from these studies are not generalizable and are considered not supportive for this application.

Of note, results of the pivotal study (including the 25 treated patients) were presented and discussed at scientific advice meeting with EMA (13 June 2019) and were considered too limited to be able to achieve conditional marketing in the targeted indication of "Treatment of pruritus associated with PFIC" (as was planned at that time). Additional data from the MRX-502 study (a double-blind, placebo-controlled and randomised study in PFIC population, that was envisaged to be completed within 9-12 months were considered necessary to support the marketing authorisation. The CHMP also emphasized that support/demonstration of clear benefit in terms of clinically relevant hard endpoints such as postponement or deferral of liver transplant would be ultimately required even if the indication were restricted to PFIC2 treatment only.

However, the applicant decided not to follow the CHMP advice and has submitted this application without data from the MRX-502 study. To meet the CHMP's request on hard clinical endpoints (deferral/delay in liver transplant), comparison of LUM001-501 data vs. natural history data from NAPPED database for EFS was provided.

Design and conduct of clinical studies

No dedicated dose-finding study was conducted and dosing recommendations are based primarily on the experience with maralixibat in the patients with PFIC2 in LUM001-501 (pivotal) study, as well as, in healthy adult volunteers and adult patients with other indications. The recommended therapeutic (280 µg/kg/day QD; equivalent to 266 µg/kg/day maralixibat free base) dose was tested in the pivotal study and may be acceptable as long as a positive benefit-risk ratio can be established. Data on the maximum recommended dose (280 µg/kg BID, i.e. 560 µg/kg/day; equivalent to 532 µg/kg/day maralixibat free base) are strictly limited (5 patients with PFIC2) and clear therapeutic benefit cannot be derived. This dose is not supported at this point in time and requires further justification. No dose-titration is proposed considering favourable tolerability of maralixibat in non-target population and as better tolerability is hypothesized in the PFIC2 population. This proposal is not supported, as extrapolation of the data from non-target population (adults, healthy/less vulnerable population, short treatment duration, different formulation of the medication applied) to the patients with PFIC2 is not possible, as the starting dose of 280 µg/kg/day was not tested in the targeted population, and as cases of downtitration due to tolerability issues were reported in the PFIC2 population. The applicant should consider introduction of up-titration phase. Further, option of use of 140 µg/kg/day (equivalent to 133 µg/kg/day maralixibat free base) as an additional therapeutic dose (e.g. as lower therapeutic dose, or in the patients with poor tolerability) should be evaluated as most of the patients responding to maralixibat appeared to have gained benefit on this dose, and since this dose was applied in the patients with tolerability issues. Thus, overall, proposed dosing regimen is not accepted. (OC)

Weight-based dosing is proposed. This approach has not been properly justified, but may be acceptable for the tested age group as long as the above open issues are satisfactorily resolved. However, extrapolation of dosing recommendations to older age groups, specifically due to the questionable weight-based dosing is not possible at this point in time. (LOQ)

Pivotal data on efficacy are provided from a single clinical study (LUM001-501) in the PFIC2 population, which was a single-arm open label Phase 2 exploratory study with small sample size and included population with PFIC1 and PFIC2 conditions. Lack of a control arm, randomisation and blinding are obvious drawbacks of the study and the sample size is regarded too small, even considering the rarity of the disease.

The study allowed enrolment of patients from age of 12 months and up-to 18 years. Notably, not all enrolled patients had genetically confirmed diagnosis of PFIC2 (and subtypes), which raises a question about adequacy of the studies population. (LoQ) Patients with elevated sBA (> 3xULN), with low GGT, without surgery of biliary diversion, concomitant decompensated cirrhosis, or any other relevant concomitant conditions were enrolled. There were no baseline pruritus eligibility criteria. Administration of bile acid or lipid binding resins, or sodium phenyl butyrate 30 days prior and during the study was prohibited. Use of medications against pruritus was restricted.

Of note, 5 protocol amendments were introduced during the study, affecting the inclusion/exclusion criteria, concomitant medication use and study medication. The study was conducted in US, UK, Poland, and France. Overall duration per participant was up-to over more than 5 years.

Key efficacy parameters were based on biomarkers of cholestasis and of liver damage. The primary efficacy endpoint was change from baseline to Week 13 in fasting sBA. sBA levels are considered a pharmacodynamic parameter for maralixibat and is regarded indicative marker of ASBT inhibition. Reductions in sBA are assumed to improve prognosis of PFIC/PFIC2 patients with improved event-free and overall survival and have positive impact on pruritus, fatigue and quality of life (van Wessel et al., 2020). Consequently, sBA, albeit not formally validated, is considered an acceptable surrogate parameter. However, demonstration of clinically relevant effects, such as improvement in pruritus, or positive effects on hard clinical endpoints (e.g. improved event-free survival, transplant-free survival) is required to confirm the benefits of maralixibat. Further, in the absence of a control group, changes in sBA from baseline can be unequivocally interpreted as causally related to treatment only if the observed changes are at a size that cannot be ascribed to natural fluctuation without treatment. As the study did not include placebo-control, interpretation of the changes in sBA is problematic. The same applies for secondary/exploratory endpoints. (LoQ)

Secondary efficacy endpoints included the change from baseline to Week 13 in pruritus, ALT, and bilirubin. Exploratory efficacy endpoints included the change over time in sBA, pruritus, ALT, bilirubin, other biochemical markers of cholestasis, and health-related QoL. The same endpoints, as well as z-scores for growth, impression of change, cholestasis biomarkers and various responder analyses were studied as exploratory parameter over long-term treatment duration.

Intractable pruritus impacts lives of the patients disturbing their sleep, leading to chronic fatigue and poor quality of life, to the extent when surgical procedures become necessary. Relief in itch is a highly clinically relevant treatment effect. Pruritus was assessed using ItchRO(Obs), a tool that was validated in the patients with Alagille syndrome. Applicability of ItchRO(Obs) in the patients with PFIC2 is currently unclear and needs to be justified. (LoQ)

Height and weight development is known to be disturbed in the patients with PFIC2. Changes in z-scores of height and weight are regarded a highly relevant and objective parameter and are supported as exploratory endpoints in the study.

Multiple definitions of responders (pre-planned and post hoc) were applied, which were mostly based on changes in sBA and ItchRO(Obs). The applicant attempts to present the responder analyses as pivotal evidence. Relevance of responder analysis is acknowledged. However, majority of these analyses were conducted post hoc with the knowledge of data and can be considered as only explorative. Comparisons between responders and non-responders has to be interpreted with high level of caution as well. Differences in baseline variables were observed between responders and non-responders which could potentially lead to bias.

The statistical methods were appropriate for an exploratory phase 2 study. The study was not planned to provide a confirmatory proof of efficacy. Several interim analyses were conducted for this single arm trial with staff directly involved in the study reviewing the results (with the possibility to change the

design), which is acceptable for an exploratory phase 2 study but problematic for a study intended to be pivotal for the application due to the potential for data-driven changes.

The study included both PFIC2 and PFIC1 patients (whereby PFIC2 patients were later subdivided in patients with nt and t mutations). Besides mentioning that summaries were to be provided by PFIC1 and PFIC2 independently and all subjects combined, no strategy was specified to take the multiplicity of patient populations into account.

Missing values were replaced by last observation carried forward. This is not appropriate, as potential effects of treatment (particularly on sBA and itching) cannot be expected to be maintained after discontinuation of treatment. Therefore, analyses should be provided where missing data are replaced in a conservative way (**LoQ**)

Baseline values for some efficacy parameters (sBA, ALT, AST, bilirubin, etc.) were represented by single measurements. In the absence of a control arm and adequate population size this is considered inadequate, as these parameters may be prone to natural fluctuations, single values may not reflect the true state of the disease at baseline. Consequently, assurance is needed that true treatment effects are being reflected in the changes from baseline derived for sBA, ALT, AST, and total/direct bilirubin. (**LoQ**)

To summarize, the study was planned as an exploratory study and the design and analyses are inadequate and insufficient to replace pivotal evidence. (MO)

Participants were up-titrated to 280 µg/kg once daily (or maximum tolerated dose) and remained on this dose till end of the study. Five patients with PFIC2 with insufficient response were allowed per protocol amendment 4 to further escalate the dose to 280 µg/kg BID in the follow-up period of the study. As already mentioned, this treatment scheme is not fully reflected in the proposed dosing regimen in the PI and the proposed dosing regimen is not acceptable. (OC)

Efficacy data and additional analyses

In total 37 patients were screened and 33 patients with PFIC (8 with PFIC1 and 25 with PFIC2) were enrolled in the study. Patients with PFIC2 had a median age of 4 years and majority had been receiving concomitant treatments in the past. It is currently unclear whether the recruited patient population is representative of general PFIC2 population, as it seems that the patients had more mild forms/less progressed form of the disease, compared to the published data. (LoQ) Also, generalisability of the data to older age groups has not been discussed and needs to be provided in order to avoid restrictions in the targeted indications. (MO)

Majority (68%) of the population with PFIC2 (17/25 patients) discontinued the study. Predominant reasons of discontinuation were refusal to continue study treatment/participation (i.e. non-compliance/stopping treatment, withdrawal of consent either by patient or caregiver, refusal to continue treatment in follow-up study; 6/25 [24%]) and disease progression/liver transplantation (6/25 [24%]; 3/19 of patients with nt-PFIC2 and 3/6 patients with t-PFIC2). Further patients with PFIC2 (5/25; 20%) withdrew due to AEs over the whole study duration of up-to more than 5 years.

High number of drop-outs due to refusal to continue the treatment (especially in the subgroup with nt-PNIF2) is assumed to reflect the patients' dissatisfaction with the received treatment and is considered relevant finding. Considerable number of cases of disease progression/liver transplant (especially in the t-PFIC2 population) is consistent with the natural data known from the literature.

The primary efficacy endpoint in PFIC population was not met. Neither was the mean numerical change in sBA at Week 13/ET in the PFIC2 population (mITT) clinically relevant.

Consistent with the primary endpoint, no relevant changes were observed in objective parameters (ALT, AST, total and direct bilirubin) at week 13 of treatment with maralixibat. Change from baseline in ItchRO(Obs) 4-week average morning score showed statistical significance, but the p-values have no confirmatory value in this trial and magnitude of numerical change is not convincing in terms of clinical relevance.

Over the long treatment period mean values of efficacy parameters revealed gradual improvement compared to baseline in the PFIC2 population. Long-term effect on primary and secondary endpoints (beyond the primary timepoint for evaluation, i.e. week 13) seem to support efficacy of maralixibat. However, this observation is misleading, as patients without response discontinued the trial and only those with response remained, thus defining the positive changes in efficacy parameters in the follow-up phase of the study. Additional info

The applicant conducted various post hoc analyses testing different definitions of responders ("multiparameter responder" based on reduction in sBA and ItchRO(Obs), "sBA responder" base on reductions in sBA only). These analyses detected 6-7 patients (out of 25 with PFIC2) who experienced improvements in sBA and pruritus during the study. Majority of these patients had normal or close to normal baseline values in AST/ALT, total bilirubin, suggestive of very mild PFIC2 type of condition. These parameters did not show relevant changes, partly due to the fact, that no elevations were reported at the baseline. All responders suffered from nt-PFIC2 type of mutation.

It is difficult to draw solid conclusions based on these analyses. It is agreed that relevant and sustained decrease in sBA and pruritus may be suggestive of treatment response. However, it is unclear which concomitant treatments were used in the responder patients and how these might have impacted efficacy (e.g. pruritus) assessments. Further, some "responder" patients displayed fluctuating sBA levels on treatment with maralixibat , that may suggest that either the effects are not durable, or there was no effect from maralixibat treatment in the first place. Finally, normal or close to normal levels of baseline AST/ALT and bilirubin, appear to indicate on a condition far milder than what has been described in the PFIC2 population (van Wessel et al., 2020). That leads to the question whether the "responder" population of LUM001-501 study is representative of general population of PFIC2/nt-PFIC2, and whether the efficacy findings observed in the "responder" subgroup can be extrapolated to broader population of PFIC2/nt-PFIC2 patients. (LOQ)

In the overall PFIC2 group (both nontruncated (nt) - and truncated (t)- PFIC2) a reduction of sBA was observed. However, there seems to be a difference in treatment effect within nt-PFIC2 patients and in some responder patients increase in sBA was observed later on treatment with maralixibat. Relevance of these observations is currently unclear and needs to be clarified. (LOQ)

Comparing z-scores for growth between responders and non-responders, showed that the responders might have had some benefit from the treatment, whereas non-responders z-scores continued worsening. Changes in z-scores are considered a relevant finding, as these scores reflect objective changes and cannot be biased through placebo effects. In this context, improvements observed in the height and weight z-scores in the "responder" group are somewhat reassuring. However, the comparison to "non-responder" population is regarded inadequate, as there was obvious difference between the "responder" and "non-responder" groups, suggesting the presence of much milder PFIC2 disease in the "responder" population. An updated analysis of z-scores with additional information on type of background mutation (BSEP1, BSEP2) in "responder" population is requested. (LoQ)

A disproportionally high ratio of responders (about half of all responders and all patients recruited by the site displayed treatment response) concentrated at 1 US site (Pennsylvania) was noted, together with the major protocol deviations (i.e. violating key selection criterion on sBA) and cases of use of forbidden medication. These findings raise concerns with regard to GCP conformity and data integrity. (LoQ)

None of the participants with t-PFIC2 form showed an effect of maralixibat, neither is efficacy considered biologically plausible, given the absence of BSEP function in these patients. Therefore, restriction of indication to patients with nt-PFIC2 population should be considered by the applicant. (MO)

The applicant analysed start of treatment effect and persistence of effects in the PFIC2 patients. Subgroup analysis of the "sBA responders" (the patients with decrease in sBA of < 102 µmol/L or by ≥ 75% compared to baseline) showed, that majority of these patients (6/7) met at least one of the 2 response criteria already at week 2 or week 4 of treatment with maralixibat. Notably, at weeks 2-4 of the treatment patients were treated with lower dose of maralixibat of 140 µg/kg/day QD. Which raises the question, whether this dose may be suitable as the therapeutic dose for those patients who respond to this dose of maralixibat, or do not tolerate 280 µg/kg/day dose.(LoQ) Thus, it seems that if true efficacy of maralixibat in responder patients exists, the effects are seen soon after start of treatment. The applicant claims that maralixibat effects were sustained. However, additional information is required to conclude on this topic.(LoQ)

Analysis of the biomarkers of bile acid synthesis (e.g., C4, FGF19) showed changes in response to maralixibat use (mean and median values of C4 increased and median values of FGF19 decreased). It is assumed that these parameters reflect increased loss of BA. Decreased levels of FGF19 are known to lead to reduced inhibition of BA synthesis. The question is raised what is the relevance of these changes and whether there is a risk of increased BA synthesis (i.e. with worsening of cholestasis) in the treated population. Additional information including e.g., a subgroup analysis in responder vs. non-responder patients is requested. (LoQ)

A post hoc sBA responder analysis on transplant-free survival (TFS) was conducted in LUM001-501 nt-PFIC2 study participants. In order to provide a complete dataset, Mirum collected follow-up information on clinical events on all, but 2 discontinued nt-PFIC2 participants who from Study LUM001-501. Per the pre-specified SAP, an analysis was conducted on maralixibat sBA response compared with maralixibat sBA nonresponse, with response defined as sBA <102 µmol/L or 75% reduction compared with baseline (as validated by van Wessel et al., 2020). Time to occurrence of liver transplant in maralixibat responders compared with nonresponders was analysed. Results from this analysis demonstrated that 100% of study participants who achieved the sBA response criteria remain transplant free, whereas the majority of participants without sBA response received a liver transplant.

The difference between the groups was statistically significant. However, due to the post-hoc nature this analysis needs to be considered as explorative. The applied responder definition was not pre-specified. Although, it is acknowledged that the definition was not data-driven but based on external data from a publication. Still, this definition was adopted in knowledge of the data. The restriction to the nt-PFIC2 population was made in knowledge of the data (but it is acknowledged that results were consistent in the overall PFIC2 population).

A comparison between responders and non-responders has to be interpreted with caution. Differences in baseline variables were observed between responders and non-responders which could potentially lead to bias. Additional confounding due to unknown or unobserved variables may also be possible. (LOQ)

Apparent baseline differences between the "responder" and "non-responder" patients in LUM001-501 suggest that "responders" might have suffered from milder form of PFIC2. Consequently, better TFS in the responder group, may not be attributed to the treatment effects. The applicant is requested to discuss potential effects of the severity of the PFIC2 in the responder and non-responder patients, or other factors which might have influenced "response" to maralixibat, including the differences in TFS. To better understand the differences between the nt-PFIC2 responders and non-responders, the

Applicant must present and discuss the clinical data (BSEP type 1 or 2, SBA, pruritus, bilirubin, ALT, AST) for these 19 patients. (LoQ)

The applicant is also requested to provide the updated analysis of TFS based on the updated follow-up information (nt-PFIC2 and t-PFIC2 patients). As the patients from the LUM001-501 study appear to have had milder form of PFIC2 when compared to the patients from the NAPPED database (van Wessel et al., 2020), applicability of the response criteria (<102 µmol/L or ≥ 75% reduction in sBA) validated as prognostic markers in NAPPED population is questioned and should also be discussed. (LoQ)

To meet the CHMP's request on hard clinical endpoints (deferral/delay in liver transplant), the applicant compared LUM001-501 data in the PFIC2 population vs. natural history controlled for event-free survival (EFS) analysis. Natural history controls were selected from the NAPPED (NAtural Course and Prognosis of PFIC and Effect of Biliary Diversion Study Group) registry, which is a non-interventional, multi-national, multi-center longitudinal registry study of approximately 700 PFIC patients of different subtypes. It is acknowledged that the registry is a retrospective database of standard of care. However, basic information about the NAPPED registry is missing and should be provided for proper evaluation of the data. The primary endpoint was a composite time to event endpoint, whereby an event was SBD, liver transplant or death, whatever occurred first. These events were considered as "hard outcomes" which is accepted. However, comparison of such hard clinical outcomes between a study and historical data can be biased and difficult to interpret, as criteria for conducting a surgery may not be objective and differ between study and historical controls, across countries, availability of alternative treatment options and valid standards of care at the time of surgical procedure. Key selection criteria from LUM001-501 study were applied to the control group from NAPPED to align the compared patient populations. The second step to ensure alignment was the selection of baseline (time '0') among those visits where a patient was eligible (according to in/exclusion criteria). This is a key issue for a time to event analysis. The selection was based on a logistic regression analysis, that is not fully comprehensible from the information provided such that it is not clear whether it ensures optimal achievable balance. Several supporting sensitivity analyses aiming to address concerns such as baseline confounding were provided. (LoQ)

Results of the primary natural history analysis (patients with nt-PFIC2) showed that the time to clinical event in the maralixibat cohort was delayed compared with NAPPED natural history controls. EFS was higher in the maralixibat cohort than in the NAPPED control group.

Pre-specified analyses with adjusted clinically relevant baseline parameters showed statistically significant differences between the maralixibat-treated group and the NAPPED control group. Notably, a precipitate drop in event free survival in the control group almost from baseline was observed, whereas there were no events reported during the first year of treatment with the test substance. This may be indicative of differences between groups rather than a drug effect. (LOQ) When controlling for mutation severity, sBA, age, ALT, AST, year of birth, and sex, the EFS advantage for maralixibat was larger. Secondary analysis that included all PFIC2 (both t-PFIC2 and nt-PFIC2) patients was consistent with the results of the sensitivity and primary analyses, suggesting improved EFS in the maralixibat-treated group, although the nominal effect size was weakened by including the t-PFIC2 patient group.

Overall, the presented data suggest favourable effects of maralixibat treatment in prevention or delay of clinically relevant events (e.g. SBD, LT). However, time to clinical event is influenced by many factors beyond treatment such that there is potential for confounding leading to bias when comparisons are made vs external data. It is acknowledged that analyses adjusting for confounding were performed to reduce bias but these analyses provide unbiased estimates only if strong unverifiable assumptions are made, such that residual bias is likely. Therefore, this analysis cannot provide pivotal evidence of an effect on delaying clinical events but can at best be considered

supportive. In particular, there are a number of concerns, which are raised and need to be addressed. (LoQ)

While the primary endpoint as such is considered clinically relevant, comparison of such hard clinical outcomes between a study and historical data can be biased and difficult to interpret. For example, the timing and type of interventions applied (liver transplant or SBD) may not always be conducted based on objective reasons (e.g. end-stage liver disease). Being in study might have influenced the decision whether and when a surgery is conducted, and time of surgery might have also depended on extrinsic factors such as organ availability, which may systematically differ between patients in the study and the registry. It is unclear whether the same criteria for deciding on interventions were used within the study and the natural history cohort. **(LoQ)**. Further, response to maralixibat treatment during the clinical study might have affected subsequent patient management, e.g. a lack of response to interruption of the enterohepatic circulation with maralixibat might have negatively influenced the decision to perform SBD in these patients. Notably, in Study LUM001-501, after nonresponse to maralixibat, all patients with undergoing surgical treatment received liver transplants, whereas in the NAPPED cohort, there was a greater proportion of SBD events. Therefore, the "time to event analysis" was mainly a comparison between time to SBD in historic controls with time to liver transplant in study LUM001-501 population. To support the interpretation of the analysis, the exact proportion of SBD events among primary endpoint events and their timing should be given for NAPPED. In addition, the clinical relevance of the different event types should be discussed and whether it is appropriate to combine these in one composite endpoint. **(LoQ)**. In addition, an analysis comparing transplant-free survival should be conducted **(LoQ).**

Further, basic information about the NAPPED registry is missing and should be provided **(LoQ)**, including information on patient selection process, on the data available in the registry and data quality. The general underlying question is in how far the patients and their course of disease that are covered in the NAPPED registry can be considered as representative for nt-PFIC2 patients and their natural course of disease.

nt-PFIC2 controls from the NAPPED registry were selected based on key inclusion and exclusion criteria for Study LUM001-501. This is a necessary but not a sufficient criterion to ensure comparability of patients from LUM001-501 and NAPPED with regard to important confounding factors. Although the restriction of the year of diagnosis to after 1990 is acknowledged, the diagnostic years of the MTX and NAPPED cohorts are largely non-overlapping such that a birth cohort effect cannot be excluded.

Selection of baseline (time '0') among those visits where a patient was eligible (according to in/exclusion criteria) is a key issue for a time to event analysis. The logistic regression analysis, utilized for baseline selection is not fully understood and additional explanation including the SAS code used for the analysis should be provided **(LoQ)**. It is not clear whether the applicant's approach leads to the optimal achievable balance of confounding factors among the available visits. In addition, even if the optimal possible balance could be achieved, this does not imply a good balance.

There may also be additional differences regarding post-baseline treatment for NAPPED patients and study patients beside MRX. Even among NAPPED patients, the treatments that are used in the patients are expected to be heterogeneous, for example because of differences between centers, changes in clinical practise with time.

Several supporting analyses adjusting for baseline covariates were provided. However, it needs to be explained why the analysis considering first eligible visit as baseline, which should be more conservative than the analysis with a random visit being considered as baseline, shows a larger treatment effect **(LoQ)**. Although the sensitivity analyses are reassuring with regard to some issues, the fundamental concerns with regards to a potential selection bias, differences between study and registry (e.g., factors potentially influencing decision and timing of surgery, reported types of events),

and residual bias due to lack of comparability between study and registry data cannot be fully resolved by these analyses.

Overall, comparison with historical controls may be relevant but requires convincing justification for the comparability of the study population and historical controls as well as justification that treatment effect is robust and the usual course of the disease highly predictable. Furthermore, historical controls should only be used when the endpoints are objective and the impact of baseline and treatment variables on the endpoint is well characterized. The Applicant should justify the appropriateness of using historical controls in this setting.(LOQ)

No further plans regarding collection of long-term follow-up data on clinical hard endpoints and additional safety data have been presented by the applicant besides the information on ongoing clinical trials. The applicant should discuss whether collection of long-term data is planned and in which form. (LOQ)

Additional expert consultation

An Expert consultation is currently not warranted.

Assessment of paediatric data on clinical efficacy

Data in children are an integral part of the study programme. This part is N/A.

Additional efficacy data needed in the context of a conditional MA

The applicant did not apply for CMA. This question is N/A.

3.3.6. Conclusions on clinical efficacy

Generally, small sized clinical databases in rare conditions are expected. However, a well-designed and conducted clinical program is also required. The submitted clinical program is considered insufficient to support the targeted indication, and concerns regarding GCP compliance are raised.

The conducted phase 2 study in PFIC2 population is not adequate to support the application. Neither are the collected data convincing and sufficient to provide pivotal evidence. Additional comparative analysis of main study data with historical control could be considered supportive, if the numerous open issues can be adequately addressed. Overall, firm conclusions on efficacy of maralixibat in the PFIC2 patient population cannot be drawn at this point in time and based on the available data. Therefore, benefit-risk assessment is not possible, and is, thus, regarded negative.

Studied population does not reflect the targeted indication, so that restrictions in the indication may be necessary, unless solid argumentation can be provided on generalisability of data to broad PFIC2 population and older age group.

Proposed dosing regimen, especially the starting dosage/absence of up-titration phase, added benefit from the maximum dosage and the proposed therapeutic dosage, has not been sufficiently justified.

3.3.7. Clinical safety

Maralixibat applies currently for the **treatment of Progressive Familial Intrahepatic Cholestasis Type 2 (PFIC2) in patients 1 year of age and older**. The product has been studied in > 1600 participants in other indications no longer followed, but only data from 119 children with cholestatic liver

disease (n = 33 with PFIC; n = 86 with ALGS) was analysed regarding the safety outcome in this submission.

The clinical program in patients with cholestatic liver diseases includes 1 Phase 1 study in overweight and obese adult participants (SHP625-101); 9 Phase 2 studies, including 5 studies in pediatric participants with ALGS (LUM001-301, -302, -303, -304, -305), 1 study in pediatric participants with PFIC1 and PFIC2 (LUM001-501), 1 ongoing study in pediatric participants with ALGS or PFIC (MRX-800), 1 study in adult participants with PBC (LUM001-201), and 1 study in adult participants with PSC (LUM001-401); as well as 2 ongoing Phase 3 studies in pediatric participants with PFIC (MRX-502 and MRX-503). (Table S1 provides an overview; for more details please refer to Efficacy section – Introduction.)

In total, from the 5 very small studies with pediatric subjects only one study includes the narrow target population of pediatric participants with PFIC1 and PFIC2 (LUM001-501), while the participants of other 4 trials (LUM001-301, -302, -303, -304, -305 as well as the ongoing trial MRX-800) suffer from ALGS.

In consequence, pooled safety from those participants receiving maralixibat in five phase 2 studies of participants with ALGS (LUM001-304, LUM001-301, LUM001-305, LUM001-302, and LUM001-303) was presented in addition.

Patient exposure

Subjects were treated for more than 5 years. Information regarding drug exposure in the different trials is currently not fully comprehensible, which might be explained by due to different study durations and dose escalations/posologies investigated in PFIC and ALGS children.

PFIC-population

In open uncontrolled SAT LUM001-501 in total 33 subjects were included, 8 participants had PFIC1 and 25 participants had PFIC2. The median treatment duration for all 33 participants is reported with 599.0 days (range: 112–2009 days). Median exposure was not fully evaluable. The study protocol defined 5 different parts with respect of dosing: a 4-week dose escalation period, a 4-week stable dosing period at 140 µg/kg/day, a 5-week stable dosing period at 280 µg/kg/day, a 59-week long-term exposure period, and an optional follow-up treatment period for eligible participants who chose to stay on treatment with MRX. During the optional follow-up treatment period, participants may have had their dose of MRX increased to a maximum of 560 µg/kg/day (280 µg/kg twice daily [BID]). It is mentioned that all 33 participants received the proposed marketed dose of 280 µg/kg once daily, of whom 10 participants subsequently received 280 µg/kg twice daily. This might be seen as equivalent to the dose recommendation given in the product information [*The recommended dose is 266 µg/kg (equivalent to 280 µg/kg maralixibat chloride) once daily, taken approximately 30 minutes before the first meal of the day, according the product information. Dose may be increased to 266 µg/kg twice daily with the second daily dose taken approximately 30 minutes before the evening meal.*]

ALGS-population

The applicant stated that of the 57 participants in the 13-week placebo-controlled studies LUM001-301 and LUM001-302, a total of 25 participants received maralixibat ≤ 140 µg/kg once daily, 14 received maralixibat 280 µg/kg/day, and 18 received placebo. The median treatment duration was 91.0 days for all participants. Although this seems rather short, it need to be considered that in the other ALGS open-label extension studies (LUM001-303, LUM001-305, and LUM001-304), the median treatment duration for the overall maralixibat group was 913.0 days and ranged from ≤ 13 weeks to > 208 weeks. Since the overall maralixibat group, the majority of participants had a treatment duration > 104 weeks (47/84

participants [56.0%]), exposure in this rare orphan paediatric disease population may be acceptable at the end. However, information on the total administered dose and the corresponding descriptions were not found and are difficult to assess due to the partially different dose escalation strategies. Thus, in general exposure relevant for the applied posology was not adequately or comprehensible assessable from the submitted documents. Thus, the applicant is requested to clarify the administered dose and provide a comprehensible overview regarding the administered doses and the total exposure in the trials in order to conclude whether the available safety data really reflect the proposed posology at the end. Moreover, the differences in exposure between the applied-PFIC and the not-applied supportive ALGS-population should be characterised and justified (OCs). Moreover, the significantly larger safety data available from about 1600 adult patients which were exposed to maralixibat was available for further assessment. Although potentially larger discrepancies due to other underlying diseases are fully acknowledged, this information is important for the safety assessment and should be submitted at least in summary documents (OC). Additionally a justification should be given that safety data for the ALGS population can be seen as supportive to enhance the paediatric safety population here applied. Differences between PFIC and ALGS in the context of the maralixibat treatment should be discussed considering that at least with respect to efficacy, data from ALGS are considered not generalizable (OC).

Adverse events

PFIC-Population (LUM001-501)

The following Table presents an overview of the general characteristics of TEAEs and safety relevant events in the applied PFIC Safety Population during the study:

Table 13 Key Safety Characteristics of PFIC subjects from the pivotal trial LUM001-501

PFIC Type: Overall	**N=33**	**N=10**	**N=33**
Participants with at Least 1:			
TEAE	33 (100.0)	10 (100.0)	33 (100.0)
TEAE Potentially Related to Study Druga	24 (72.7)	5 (50.0)	26 (78.8)
Serious TEAE	15 (45.5)	0	15 (45.5)
Serious TEAE Potentially Related to Study Druga	5 (15.2)	0	5 (15.2)
TEAE Leading to Study Drug Discontinuation	9 (27.3)	1 (10.0)	10 (30.3)
TEAE Leading to Death	0	0	0
PFIC Type: PFIC1	**N=8**	**N=5**	**N=8**
Participants with at Least 1			
TEAE	8 (100.0)	5 (100.0)	8 (100.0)
TEAE Potentially Related to Study Druga	4 (50.0)	3 (60.0)	6 (75.0)
Serious TEAE	4 (50.0)	0	4 (50.0)
Serious TEAE Potentially Related to Study Druga	1 (12.5)	0	1 (12.5)
TEAE Leading to Study Drug Discontinuation	0	1 (20.0)	1 (12.5)
TEAE Leading to Death	0	0	0
PFIC Type: PFIC2	**N=25**	**N=5**	**N=25**
Participants with at Least 1			
TEAE	25 (100.0)	5 (100.0)	25 (100.0)
TEAE Potentially Related to Study Druga	20 (80.0)	2 (40.0)	20 (80.0)
Serious TEAE	11 (44.0)	0	11 (44.0)
Serious TEAE Potentially Related to Study Druga	4 (16.0)	0	4 (16.0)

TEAE Leading to Study Drug Discontinuation	9 (36.0)	0	9 (36.0)
TEAE Leading to Death	0	0	0

Source: LUM001-501 Addendum CSR, Appendix 8.1, Table 14.3.2.1.
AE = adverse event; BID = twice daily; n = number in a given category; MRX = maralixibat; N = number of participants; PFIC = progressive familial intrahepatic cholestasis; QD = once daily; TEAE = treatment- emergent adverse event.

The most frequent treatment-related TEAEs were similar frequent: diarrhea (13 participants [39.4%]), abdominal pain (9 participants [27.3%]), abdominal pain upper (6 participants [18.2%]), vomiting (5 participants [15.2%]), and INR increased (5 participants [15.2%]). In 6.1% of the subjects (n=2/33) hyperbilirubinaemia was observed.

ALGS-population (LUM001-304,301/302, long-term population)

All participants in LUM001-304 had at least 1 TEAE. In the ALGS Study LUM001-304, during the 18-week open-label period, 30/31 participants (96.8%) experienced at least 1 AE. Twelve participants (38.7%) experienced an AE potentially related to study drug. A total of 4 participants (12.9%) experienced an SAE; none was considered potentially related to study drug.

During the randomized withdrawal (RWD) Period of the LUM001-304 study, **fewer participants randomized to maralixibat 400 µg/kg/day (7/13, 53.8%) experienced an AE than those randomized to placebo (12/16, 75.0%)**.

One participant (7.7%) receiving maralixibat 400 µg/kg/day experienced an AE potentially related to study drug compared with 3 (18.8%) participants receiving placebo. One participant in each group experienced an SAE during the RWD Period; neither SAE was considered potentially related to study drug.

This is in accordance with the reports from the pooled 13-week controlled studies (LUM001-301 and 302) in ALGS were TEAEs were rather similar and overall not additional informative. In both groups subjects experienced at least 1 AE (M: 89,7%/ P:88,9%).

During the open-label, long-term extension studies, 83/84 (98.8%) participants in the overall maralixibat group experienced at least 1 AE. Approximately 63% of participants had AEs considered potentially related to maralixibat; proportions of potentially related AEs ranged from 58.3% (> 280 µg/kg/day group) to 70.0% (≤ 140 µg/kg once daily group).

During the approximate 5-year duration of these studies (data cutoff date: 01 Dec 2019), 25/84 (29.8%) participants experienced at least 1 SAE. The highest proportion of SAEs occurred in participants receiving > 280 µg/kg once daily maralixibat. However, only 3 (3.6%) participants experienced an SAE considered potentially related to maralixibat; 2 participants received maralixibat 280 µg/kg once daily and 1 participant received maralixibat ≤ 140 µg/kg once daily. **In summary, there seemed to be a trend for more TEAEs and SAEs in subjects > 280 µg/kg once daily maralixibat as far as this can be evaluated in the small population available.**

The most common TEAEs (using a threshold of > 10%) observed across the entire maralixibat program (PFIC and ALGS) were from the SOCs of GI disorders, general disorders and administration site conditions, and infections and infestations. The following preferred terms were reported as most common TEAEs (> 10% overall) in every study:

- Abdominal pain
- Cough

• Diarrhoea

• Headache

• Nasopharyngitis

• Pyrexia

• Upper respiratory tract infection

• Vomiting

Review of data in participants across the clinical development program (PFIC and ALGS; N = 119), revealed that median time to first onset for events of diarrhea and abdominal pain was 30 days and 61 days, respectively. The duration of the gastrointestinal events were short as reflected by a median duration for events of diarrhea and abdominal pain were 2 days and 1 day, respectively. The majority of these events were mild to moderate in severity, transient in nature, and resolved with no action taken with maralixibat.

Treatment-Related Adverse Events

Treatment-emergent AEs potentially related to study drug were experienced by 26 participants (78.8%) overall, including 6 participants with PFIC1 (75.0%]) and 20 participants with PFIC 2 (80.0%). Serious TEAEs were experienced by 15 participants (45.5%) overall, including 4 participants (50.0%) with PFIC1 and 11 participants (44.0%) with PFIC2.

PFIC-Population in Study LUM001-501:

Any TEAE determined to be possibly related or related by the investigator was categorized as related to study drug. Table 26 displays TEAEs judged to be potentially related to study treatment that were reported in 2 or more participants during the study. Treatment-related TEAEs were experienced by 26 participants (78.8%) during the study. **The most common treatment-related TEAEs included diarrhea (13 participants [39.4%]), abdominal pain (9 participants [27.3%]), abdominal pain upper (6 participants [18.2%]), vomiting (5 participants [15.2%]), and INR increased (5 participants [15.2%]).**

Table 14 Incidence of Treatment-Related Treatment-Emergent Adverse Events in 2 or More Participants During the Study (Safety Population) – Study LUM001-501 (PFIC-Population)

System Organ Classa Preferred Term	280 µg/kg QD (N=33) n (%)	280 µg/kg BID (N=10) n (%)	Any Dose (N=33) n (%)
Number of Participants with at Least 1 Treatment-related TEAE	**24 (72.7)**	**5 (50.0)**	**26 (78.8)**
Gastrointestinal disorders			
Diarrhoea	11 (33.3)	2 (20.0)	13 (39.4)
Abdominal pain	8 (24.2)	1 (10.0)	9 (27.3)
Abdominal pain upper	6 (18.2)	0	6 (18.2)
Vomiting	5 (15.2)	0	5 (15.2)
Frequent bowel movements	4 (12.1)	0	4 (12.1)
Faeces pale	2 (6.1)	0	2 (6.1)
Hepatobiliary disorders			
Hyperbilirubinaemia	2 (6.1)	1 (10.0)	2 (6.1)

System Organ Classa Preferred Term	280 µg/kg QD (N=33) n (%)	280 µg/kg BID (N=10) n (%)	Any Dose (N=33) n (%)
Investigations			
International normalised ratio increased	5 (15.2)	1 (10.0)	5 (15.2)
International normalised ratio abnormal	2 (6.1)	0	2 (6.1)
Vitamin E decreased	2 (6.1)	0	2 (6.1)
Metabolism and nutrition disorders	2 (6.1)	0	2 (6.1)
Decreased appetite	2 (6.1)	0	2 (6.1)
Psychiatric disorders			
Irritability	2 (6.1)	0	2 (6.1)
Skin and subcutaneous tissue disorders	2 (6.1)	1 (10.0)	3 (9.1)
Pruritus			

Source: LUM001-501 Addendum CSR, Appendix 8.1, Table 14.3.2.4.
AE = adverse event; BID = twice daily; MedDRA = Medical Dictionary for Regulatory Activities; n = number in a given category; N = number of participants; QD = once daily; TEAE = treatment-emergent adverse event.
a Adverse events were coded using MedDRA version 22.1.
Note: Percentages are 100*n/N. The 280 QD dose included all QD doses ≤ 280 µg/kg. The 280 BID dose included the dose of 420 µg/kg daily for 2 weeks as part of a dose-escalation. For each MRX dose group, the percent of participants was derived using a denominator based on the number of participants that received the given dose. In presenting AEs by MRX dose, events were only counted in the MRX dose group in which the start of the event occurred. Participants were counted only once for each System Organ Class and Preferred Term. Treatment-emergent adverse events are 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 participants with > 14 days of study drug interruption/withdrawal, the definition of a TEAE considers both the date of the last dose prior to drug interruption and the actual last dose.

Adverse Events of Special Interest (AESI)

According the known potential class effects with respect to use of ASBT/IBAT inhibitors the applicant has identified diarrhea, elevated transaminases, FSV deficiency, and elevated bilirubin as potential adverse events of special interest. The AESIs were based identified on broad groups of preferred terms that might have attribution to the following types of events for which a causal relationship cannot be excluded or is likely: diarrhea events, elevated transaminases events, FSV deficiency events, and elevated bilirubin events. It is stated that the designation for an event as an AESI was based solely on the reported term and did not require laboratory confirmation, which seems controversial since elevated transaminases and bilirubin as well as FSV deficiency can only be confirmed by laboratory measurements.

Serious adverse events and deaths

In the PFIC target population included in study LUM001-501 about half of the participants (45.5%) had an **SAE**. SAEs of diarrhoea, abdominal pain, and gastroenteritis were the only events (based on PT) that were reported in more than 1 participant (each SAE was reported in 2 participants; 6.1%). 5 of these SAEs (15.2%, all in 280µg/kg QD) were classified as potentially drug-related. However, without sufficient information it seems difficult to evaluate this classification, since drug-related TEAEs/SAEs are in general difficult to differentiate from disease-associated events due to the mainly gastrointestinal adverse events/toxicity of maralixibat.

The incidence of SAEs in the ALGS open-label, long-term extension studies was 29.8% (25/84 participants) for participants exposed to maralixibat. The majority of these SAEs were from the SOCs

of Infections and infestations and GI disorders; vomiting was the only SAE (based on PT) that was reported in more than 1 participant (reported in 2 participants [2.4%]). No specific patterns or safety signals were identified based on review of SAEs.

No **death** event occurred during the clinical development in the paediatric population.

Since the assessment of drug-relationship of the SAEs in both populations (PFIC/ALGS) was somewhat hampered due to the mode of reporting, the applicant is requested to provide a comprehensible analysis and evaluation of all potentially drug-related SAEs occurred in both populations (PFIC and ALGS). Sufficient details are expected (OC).

Laboratory findings

Haematology: With respect to clinical laboratory evaluations of haematological parameters in both populations (PFIC and ALGS) the mean changes from baseline in hematology parameters were minimal, short lasting and probably resolved at the next study visit. TEAEs with respect to this class were reported in about 3%. Even if a relationship would be considered, the abnormalities would indicate only a very low risk according the data. In particular, since there seems to be no apparent pattern to the changes over time. However, the applicant is requested to confirm that all abnormalities registered as clinical laboratory abnormality regarding haematology during the clinical trials were all fully resolved and the estimated duration of the TEAEs reported. Moreover, additional clarification for several other details regarding haematology parameter TEAEs observed is requested (OCs)

Serum Transaminases and Hepatic Safety:

With respect to the **hepatic safety**, laboratory data showed probably isolated, asymptomatic elevations in ALT in some ALGS participants. Whether these events have to be seen as part of the natural history of ALGS and were not associated with concomitant rises in bilirubin as the applicant concludes remains uncertain. **Although no ALT elevations were observed in the PFIC subjects and the applicant claims that improvements were observed in liver biochemistries in the sBA responders, the conclusion of the involved independent liver safety regarding the absence of an intrinsic risk for hepatotoxicity needs confirmation.** Moreover, the applicants statement that there were no reported cases of drug-induced liver injury across the clinical development program in > 1600 adult participants cannot be further evaluated, since no results were submitted. **Since the mode of reporting of the results is also not satisfying, additional clarification regarding the hepatotoxicity issue and the details of the monitoring remains needed (OC).**

Fat-Soluble Vitamins, Coagulation, and Lipid Panel: With few grade 1 exceptions, the majority of participants in the Study LUM001-501 had sufficient 25-hydroxyvitamin D levels, INR, retinol: RBP molar ratio, and vitamin A levels, and a sufficient alpha tocopherol/total lipids ratio from Week 132 onward. The mean changes from baseline in FSV, coagulation, and lipid parameters were also only minimal during the 18-week open-label period of Study LUM001-304 in ALGS. After Week 22 in the overall maralixibat group, there was no pattern of increasing proportions of participants with abnormalities among these parameters over time.

Vitamin deficiency, particularly FSV deficiency, is common in children with chronic liver diseases; this may be explained by the impaired FSV uptake, reduced food intake and reduced synthesis of carrier proteins caused by these patients' damaged liver function. According to literature data the incidence of vitamin deficiency could be 20% to 30% in patients with cholestatic liver disease as in the target population.

Nevertheless, the applicant should clarify whether and particularly how FSV-Substitution was regularly performed in the clinical trials and justify the statement concerning FSV in the product information is sufficient (OC).

Vital Signs and Other Safety Evaluations: Changes from baseline in vital signs were minimal in Study LUM001-501 and across the ALGS integrated studies and no pattern was identified which could be interpreted as a potential signal from the analyses. Moreover, since the drug is not absorbed it seems plausible that no concerns for QT prolongation risk following maralixibat administration were raised in the clinical program.

Safety in special populations

This product is applied for a paediatric population only; nevertheless the applicant stated that in total more that 1700 subjects were exposed in other indications. However, this information is not available at present, but should be (OC).

The applicant has provided subpopulation analysis for participants from Study LUM001-501 and the ALGS-integrated population. However, they were only performed regarding age (< 2 years, 2 to < 6 years, 6 to < 12 years, and 12 to 18 years) and gender subgroups. It is concluded correctly that the analyses of the data did not reveal a specific subpopulation to be notable for a preponderance of specific events/increase in severity of a particular event. However, the impact on body-weight on safety was not addressed which is particularly important in a paediatric population as applied. This should be performed (OC). Considering the small number in the subgroups the statement that no specific patterns or safety signals were identified based on review of the subpopulations remains less informative at the end.

Immunological events

No information regarding immunological events was submitted nor was this issue discussed in the documents. Moreover, no specific investigation regarding this issue were reported. Although currently there was no signal detected probably indicating such adverse events (anaphylactic reactions or other suspect events like urticaria are not reported), the applicant is requested to report, whether any data is available, which would allow assessing the allergenic potential of maralixibat further. Moreover, it needs clarification whether risk for topical immunisation was studied and whether the events of rash may reflect a signal for allergy. (OCs)

Safety related to drug-drug interactions and other interactions

Since maralixibat chemical structure is designed to be minimally absorbed following oral administration because the site of action is within the lumen of the GI tract, is metabolically stable in vivo, excreted almost exclusively in the feces as intact parent drug, it seems plausible that the drug has only a low potential for drug-drug interactions in general. Moreover, the applicant stated that no safety concerns regarding drug interactions with maralixibat were identified during the trials. Thus, it is agreed that, considering the very low plasma drug levels for maralixibat at therapeutic doses (often below the LLOQ), and also based on in silico modeling and clinical DDI studies, drug interactions with maralixibat are unlikely. However, the impact of maralixibat on absorption of FSV should be clarified more in detail and the approach, how this adverse event was studied systematically in the clinical studies, should be detailed.(OC)

It is agreed that currently there is no evidence that maralixibat has any risk for drug abuse or with respect to the ability to drive or operate machinery or leads to an impairment of mental abilities. Several cases of drug overdose occurred, but were not associated with any TEAEs during the clinical development program. Moreover, there were no apparent treatment-related withdrawal effects except for a return of pruritus among participants who stopped treatment with maralixibat.

Discontinuation due to AES

In the applied target population, a total of 10 participants (30.3%) had an AE that resulted in discontinuation of maralixibat in trial LUM001-501. 3 of the 10 (9.1%) had an event considered possibly related to maralixibat, and 7 (21.2%) had an event considered unlikely or not related to maralixibat. A total of 9 participants (27.3.%) were from the 280-µg/kg once daily group, whereas 1 participant was from the 280 µg/kg twice-daily group. Events of blood bilirubin increased (4 participants [12.1%]) and disease progression (2 participants [6.1%]) were the main reasons for treatment discontinuation in this trial.

Review of the safety data for the ALGS integrated population demonstrated that AEs leading to discontinuation of maralixibat occurred mainly during the long-term extension period of the studies.

Of the 39 participants in the 13-week, placebo-controlled studies, 2 participants had an event that led to discontinuation; 1 participant (4.0%) in the maralixibat ≤ 140 µg/kg group had an event of ALT increased and 1 participant (5.6%) in the placebo group had an event of abnormal behaviour.

Of the 84 participants in the open-label, long-term extension studies, a total of 13 participants (15.5%) had an AE that led to discontinuation.

ALT increased was the most commonly reported event that led to discontinuation of maralixibat, which included 6 participants (7.1%) from the long-term extension studies. Whereas a causal attribution with maralixibat was considered for some of these events, the events may have also been consistent with the natural history of the ALGS and progression of the underlying disease. In order to overcome the difficulties in the assessment of causality regarding a potential intrinsic hepatotoxicity, the applicant is requested to provide the safety information available from the non-paediatric population with liver diseases and hypercholesterinaemia (OC).

Post marketing experience

Currently the product is not approved anywhere. Thus, no post-marketing experience is available at present.

3.3.8. Discussion on clinical safety

Maralixibat applies currently for the treatment of the rare cholestatic liver disease "Progressive Familial Intrahepatic Cholestasis (PFIC) Subtyp 2 in patients 1 year of age and older". The proposed dose is 280 µg/kg once daily. Maralixibat is a minimally absorbed oral agent that is designed to maximize local exposure of the molecule to its target and minimize systemic exposure.

The maralixibat clinical development program in children with cholestatic liver diseases includes 3 studies in participants with PFIC (LUM001-501 in Phase II, MRX-502 and MRX-503 in Phase III), and 1 long-term study (MRX-800) that includes participants with PFIC who had completed phase II-III studies. However, data from MRX-502 and MRX-503 has not been submitted in this application.

While efficacy assessment is based on the results from the pivotal trial LUM001-501, assessment of safety includes also data from children suffering from Alagille Syndrome (ALGS), another rare cholestatic liver disease in the paediatric population. Safety data is available from 4 trials (LUM001-304, as well as 301/302 and long term extension trial 305). However, the most relevant safety population for maralixibat is restricted on 33 paediatric participants with PFIC of whom only 25 participants had PFIC2. They have been studied in pivotal (Phase II) Study LUM001-501 and the associated ongoing long-term rollover study (MRX-800), with more than 5 years of maralixibat treatment.

A justification should be given, that the additional safety data for the ALGS population can be pooled and seen as supportive to enhance the paediatric safety population here applied. Thus, differences between PFIC and ALGS in the context of the maralixibat treatment should be discussed (OCs). Given the limited number of patients no robust conclusions on safety can be drawn at present. For this reason, the update from ongoing MRX-800 study and new data from ongoing PFIC studies is of interest (OC). Furthermore, the Applicant should discuss and commit to collecting additional safety data both in terms of number of patients as well as in time **(OC)**.

Moreover, since the product was investigated in the indications of Primary Biliary Cholangitis (PBC), Primary Sclerosing Cholangitis (PSC) and hypercholesterolemia in ~ 1600 adult patients, additional data seems to be available, but was not presented. Although the difference in clinical presentation in other diseases in an adult population may complicate the assessment, this safety data should be available for comparison and discussion of rare events and exclusion of an intrinsic hepatotoxicity of maralixibat (OC).

Moreover, a comparison of the safety assessment approach in the 5 paediatric studies is requested in order to confirm validity of the presented data (OC).

Exposure

Data from 119 children with cholestatic liver disease (n = 33 with PFIC; n = 86 with ALGS) were analysed regarding the safety outcome for this submission. Subjects have been treated for up to more than 5 years. However, information regarding drug exposure in this application is currently not fully comprehensible, which might be explained due to differences in posology/dose escalation strategies investigated in PFIC and the ALGS study populations and other reasons. Nevertheless, the cumulative daily doses and other exposure data should be provided (OC).

Considering limitation in the safety-database because paediatric cholestatic diseases as PFIC and ALGS are rare orphan diseases, exposure might be nevertheless probably seen as acceptable at the end.

Adverse events

All patients in the safety population experienced TEAEs and 78.8% of these events are evaluated as potentially related to study drug and 15.2% of serious TEAEs were potentially related to study drug.

Adverse events in the GI SOC were reported in 84.8% of participants. The other TEAEs that occurred at a rate of > 50% were from the SOCs of general disorders and administration site conditions, infections and infestations, respiratory, thoracic and mediastinal disorders and none of them were considered to be treatment related.

In accordance with the known safety profile of the pharmacological class of ASBT inhibitors GI events including diarrhea (57.6%) and abdominal pain (45.5%) were the most frequently reported adverse drug reactions for maralixibat for the applied PFIC target-population included in study LUM001-501. The majority of these events were described to be mild to moderate in severity, transient in nature, and resolved with no action taken with maralixibat and no special approaches for monitoring were required. Specific mitigation activities for these events included provision of dosing modification guidelines for GI symptoms, including diarrhea and abdominal pain, within the clinical study protocols. These

recommendations concerned dose up and down-titration and should be adequately reflected in the dosing recommendations (OC).

Safety data in the other paediatric population of ALGS subjects from the 13-week, placebo-controlled studies (LUM001-301 and LUM001-302) revealed a similar incidence of events of diarrhea in the overall maralixibat and placebo groups (43.6% vs. 44.4% of participants, respectively), while events of abdominal pain were slightly rarer reported in M: 25.6% versus P: 16.7%, respectively.

These results may indicate that GI symptoms are also likely to be present among the underlying patient population of pediatric cholestasis as disease complication. Most common TEAEs (> 10% overall) in every study (including long term exposure) were abdominal pain (45,5%), cough (34.5), diarrhea (39.4%), headache (20.7%), nasopharyngitis (34.5%), pyrexia (48.3), upper respiratory tract infections (20.7%) and vomiting (37.9%). (e.g. from LUM001-304; ISS Table 2.1.3.2.)

Specific in the PFIC-population, the most frequent treatment-related TEAEs were similar frequent: diarrhea (13 participants [39.4%]), abdominal pain (9 participants [27.3%]), abdominal pain upper (6 participants [18.2%]), vomiting (5 participants [15.2%]), and INR increased (5 participants [15.2%]). In 6.1% of the subjects (n=2/33) hyperbilirubinaemia was observed.

Insofar, diarrhea is an important identified risk for patients using maralixibat and further information on this topic is requested for inclusion in SmPC (OC). Current or sequelae from chronic diarrhea requiring specific intravenous fluid or nutritional intervention was an exclusion criterion in the pivotal study and this information should also be included in the SmPC under section 4.4 as population not studied in clinical trials (OC). Moreover, it was not adequately possible to assess the criteria for the classification of TEAEs/SAEs as drug related in both paediatric populations (PFIC/ALGS), thus, the approach should be justified comprehensible. (OC)

Review of data in participants across the clinical development program (PFIC and ALGS; N = 119), revealed that median time to first onset for events of diarrhea and abdominal pain was 30 days and 61 days, respectively. The duration of the gastrointestinal events were short as reflected by a median duration for events of diarrhea and abdominal pain were 2 days and 1 day, respectively. The majority of these events were mild to moderate in severity, transient in nature, and resolved with no action taken with maralixibat.

Moreover, it needs to be taken into account that infection events, including upper respiratory tract infection and nasopharyngitis, in general are frequent in this population. It is documented in the literature that children experienced a median of 14 simple infections during the first 3 years of life, with 71% being respiratory infections, followed by gastrointestinal infections (Vissing et al. 2018). The limited placebo-controlled data (LUM001-301/302) demonstrate at least for ALGS population that TEAE events are rather similar for maralixibat and placebo. Insofar, in summary a mild to moderate increased risk for gastrointestinal events may be presumed; however, became not clearly demonstrated at least during the 13 weeks of placebo comparison. Nevertheless, a concern exists regarding rare events and a risk for a potential intrinsic hepatotoxicity (further discussed in the laboratory section below). In addition, the overall reliability of the safety data may be challenged due to the overall low number of patients investigated, the limited placebo control data and the fact that hidden differences between the applied PFIC and ALGS population are not evaluable from this data (OCs) Therefore, it seems important to have additional analyses from the significantly larger adult population, though differences may be difficult to interpret due to the other underlying disease, at least for the assessment of gastrointestinal- and potential hepatotoxicity this data should be available (OCs).

Specific mitigation activities for these events included provision of dosing modification guidelines for GI symptoms within the clinical study protocols. Vomiting, diarrhea and abdominal pain should be described adequately as important identified risk in SmPC and RMP (OC).

Similarily, any condition impairing GI motility or small intestine absorption could impact treatment efficacy. Since maralixibat is a potent, highly selective ASBT inhibitor. ASBT is localized on the luminal surface of ileal enterocytes, and is required for mechanism of action of maralixibat. Therefore, this information should also be included in the SmPC under section 4.4 (OC).

SAEs and deaths

In PFIC population included in Study LUM001-501 about half of the participants (45.5%) had an SAE. **SAEs** of diarrhea, abdominal pain, and gastroenteritis were the only events (based on PT) that were reported in more than 1 participant (each SAE was reported in 2 participants; 6.1%). 5 of these SAEs (15.2%, all in 280µg/kg QD) were classified as potentially drug-related. However, without sufficient information it seems difficult to evaluate this classification, since drug-related TEAEs/SAEs are in general difficult to differentiate from disease-associated events due to the mainly gastrointestinal toxicities of maralixibat.

The incidence of SAEs in the ALGS open-label, long-term extension studies is reported slightly lower with about 29.8% (25/84 participants) for participants exposed to maralixibat. The available limited placebo comparison from LUM001-301/302) seems not to indicate a significant difference regarding SAEs: (LUM001-301 and LUM001-302). For example, events of diarrhea rates of 43.6% versus 44.4% of participants from the overall maralixibat group and placebo group were reported; whereas, events of abdominal pain were reported in 25.6% versus 16.7%, respectively. The majority of these SAEs were from the SOCs of Infections and infestations and GI disorders; vomiting was the only SAE (based on PT) that was reported in more than 1 participant (reported in 2 participants [2.4%]). No specific patterns or safety signals were identified based on review of SAEs.

From the submitted documents, it was not possible to fully assess the drug-relationship of the SAEs in both populations (PFIC/ALGS); the applicant is requested to provide a comprehensible analysis and evaluation of potentially drug-related SAEs for both populations (PFIC and ALGS) with sufficient details (OC).

No **death** event occurred during the clinical development in the paediatric population.

Laboratory findings:

With respect to clinical laboratory evaluations of **haematological parameters** in both populations (PFRIC and ALGS) the mean changes from baseline in hematology parameters were minimal, short lasting and probably resolved at the next study visit. TEAEs with respect to this class were reported in about 3%. Even if a relationship would be considered, the abnormalities would indicate only a very low risk according the data. In particular, since there seems to be no apparent pattern to the changes over time. Nevertheless, the applicant is requested to confirm that all abnormalities registered as clinical laboratory abnormality regarding haematology during the clinical trials were fully resolved and the estimated duration of the TEAEs reported. Moreover, additional clarification for several other details regarding haematology parameter TEAEs observed is requested (OCs).

With respect to the **hepatic safety**, laboratory data showed probably isolated, asymptomatic elevations in ALT in some ALGS participants. Whether these events have to be seen as part of the natural history of ALGS and were not associated with concomitant rises in bilirubin as the applicant concludes remains uncertain. Although no ALT elevations were observed in the PFIC subjects and the applicant claims that improvements were observed in liver biochemistries in the sBA responders, the conclusion of the involved independent liver safety regard the absence of an intrinsic risk for hepatotoxicity needs confirmation. Moreover, the applicants statement that there were no reported cases of drug-induced liver injury across the clinical development program in > 1600 adult participants cannot be further evaluated, since no results were submitted. Furthermore, the issue that during the long-term extension trials 6 subjects discontinued due to ALT increases assessed as potentially drug-related, is also not reassuring. In

addition, it needs to be discussed that several patients in LUM 001-501 study also discontinued due to bilirubin increase. Overall this PT was the most frequent AE that led to discontinuation in the whole safety population.

Since the mode of reporting of the results is also not satisfying, additional clarification regarding the hepatotoxicity issue and the details of the monitoring remains needed (OC).

None of the participants with PFIC or ALGS of childbearing age in the safety studies became pregnant. Patients who are pregnant or lactating or who are planning to become pregnant were excluded. Non-clinical reproductive and developmental studies did not demonstrate genotoxic potential or issues with neonatal development.

Subgroup analyses:

The applicant has provided some attempts for subgroup analyses regarding gender and age subgroups (< 2 years, 2 to < 6 years, 6 to < 12 years, and 12 to 18 years). In general, they are less informative for both populations (PFIC and ALGS). Due to the small number of subjects in the cohorts differences and potential trends remain not interpretable at the end. Nevertheless the applicant should provide at least the standard subgroup safety analyses. In particular, data regarding the impact of body weight on safety events remains missing; however, the applicant is requested to provide this information in addition, since is particularly important in a paediatric population as here applied (OC). Moreover, in the SCS document p143 it is reported that events of elevated bilirubin occurred in 2/11 (18.2%) participants in the < 2 years age group, and in 1/23 (4.3%) participant in the ages 6 to < 12 years age group of the open-label, long-term Extensions Cohort in ALGS subjects. The applicant is requested to report the details and further development in these cases (including the narratives), which could not be identified. (OC)

Immunological events:

No information regarding immunological events was submitted nor was this issue discussed in the documents. Although there seems to be no signal probably indicating such adverse events (anaphylactic reactions or other suspect events like urticaria are not reported), the applicant is requested to report, whether any data is available, which would allow assessing the allergenic potential of maralixibat further. Moreover, it needs clarification whether risk for topical immunisation was studied and whether the events of rash may reflect a signal for allergy. (OCs)

DDI and other potential interactions

Since maralixibat chemical structure is designed to be minimally absorbed following oral administration because the site of action is within the lumen of the GI tract, is metabolically stable in vivo, excreted almost exclusively in the feces as intact parent drug, it seems plausible that the drug has only a **low potential for drug-drug interactions** in general.

The applicant stated that based on the safety data to date (01 Dec 2019), no safety concerns regarding drug interactions with maralixibat were identified.

Thus, it is agreed that, given the very low plasma drug levels for maralixibat at therapeutic doses (often below the LLOQ), and also based on in silico-modeling and clinical DDI studies, systemic drug interactions with maralixibat are unlikely.

However, the impact of maralixibat on absorption of FSV should be clarified more in detail and the approach, how this adverse event was studied systematically in the clinical studies, should be detailed.(OC)

It is agreed that currently there is no evidence that maralixibat has any risk for drug abuse or with respect to the ability to drive or operate machinery or leads to an impairment of mental abilities. Several cases of drug overdose occurred, but were not associated with any TEAEs during the clinical development program. Moreover, there were no apparent treatment-related withdrawal effects except for a return of pruritus among participants who stopped treatment with maralixibat.

Discontinuation due to AEs

In the applied PFIC target population 10/33 participants (30.3%) experienced TEAEs that led to permanent treatment discontinuation in trial LUM001-501. Three TEAEs that led to permanent treatment discontinuation were considered as potentially related to study drug (pruritus, pancreatitis, and vitamin E decreased). A slightly lower rate was observed in the ALGS population (range 13.8% - 21.4 %); however due to the small number of subjects this difference has not inevitable to be interpreted as a real difference between the both populations. **During the long-term extension trials 6 subjects discontinued due to ALT increases assessed as potentially drug-related. Overall this PT was the most frequent AE that led to discontinuation in the whole safety population. Considering the underlying disease, this issue may reflect both the progression of the disease as well as an intrinsic mild hepatotoxicity**. Since conventional methods in assessing drug associated hepatotoxicity failed in this entity, it is essential to have additional data from the 1600 adult patients treated with maralixibat for other indications for a comparison (OC). Although the applicant stated that the incidence of AEs leading to discontinuation appeared to decrease over time, this finding is not reassuring; since it is a normal consequence of selection and insofar not surprising.

Currently the product is not approved anywhere. Thus, **no post-marketing experience is available** at present.

Additional expert consultation

Not to define at the present stage of assessment.

Assessment of paediatric data on clinical safety

N/A

Additional safety data needed in the context of a conditional MA under exceptional circumstances

Not to define at the present stage of assessment.

3.3.9. Conclusions on clinical safety

Assessment of safety in this application is hampered due to rare orphan disease nature of PFIC as reflected by the fact that data from only 33 children is available; only 25 of these can be assigned to the applied PFIC subtype 2 population. The applicant has tried to overcome this limitation by submitting additional safety data from 86 children with ALGS, another cholestatic liver disease in the paediatric population. The acceptance of mixing up these two populations for safety assessment needs more clarification.

Although it seems that maralixibat in general was tolerated and might have an acceptable safety profile in patients with paediatric cholestasis as PFIC2, a significant degree of uncertainty remains at present.

Moreover, the submitted information was partially not informative and the approach of safety monitoring in the 5 small trials seems critical and details needs further clarification.

Gastrointestinal adverse events/toxicity as diarrhea, abdominal pain and vomiting is reported to be the most common adverse drug reactions, however may be also caused by the underlying disease. Since almost of the TEAEs observed were mild to moderate and resolved with no action taken with maralixibat, no safety major objections have to be raised at the current stage of assessment. Nevertheless, it needs further clarification whether maralixibat has an intrinsic hepatotoxicity. Additional analyses and more information of the safety outcome in 1600 adult patients treated with this product may be helpful. Moreover, the product information should describe risks more detailed as currently proposed.

3.4. Risk management plan

The applicant provided an RMP version 0.1 for maralixibat chloride. Data-lock point was 3rd December 2019; date of the final sign-off was 23rd Aug 2020. The RMP refer to one product.

3.4.1. Safety Specification

Summary of safety concerns

The applicant did not propose a summary of safety concerns in the RMP.

Table SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	<List>
Important potential risks	<List>
Missing information	<List>

3.4.2. Discussion on safety specification

The applicant considered diarrhoea not important to be included as risk. However, it is not clear if these conclusions are based on the clinical programme in adults, of a pooled analysis of all patient population or PFIC patients only. Generally, in small children, that is supposed to be the target population, diarrhoea and subsequent dehydration is of concern. Depending on the applicant's response to this issue in the clinical safety part the applicant should consider including "Clinically significant or severe diarrhoea leading to dehydration and electrolyte imbalance" as important potential risk.

With regard to elevated liver enzymes, apparently elevations in transaminases were observed in non-clinical studies as well as in clinical trials with ALGS patients but not in patients with PFIC. Considering the extremely low sample size of clinical trials in PFIC patients the latter is not reassuring. Considering close monitoring of paediatric patients is routine clinical practice it should be possible to gather additional information on the matter. The applicant stated that abnormalities like those observed on maralixibat will be addressed with standard of care. The applicant should clarify what this actually means with regard to maralixibat treatment. If interrupting or discontinuing treatment this the way of addressing these abnormalities this should be clearly addressed in the product information as routine risk minimisation. Depending on the applicant´s response to this issue in the clinical safety part the applicant should consider including "Hepatotoxicity" as an important potential risk.

The applicant stated that it can be excluded that patients will use bile acid or lipid binding resins concomitantly to maralixibat. As it is not comprehensible why this co-medication can be excluded but an additive effect may cause malabsorption of concomitantly administered lipophilic substances like vitamins the applicant should consider adding "malabsorption of fat-soluble drugs, food components and vitamins" as an important potential risk in the list of safety concerns.

In light of the low number of patients and very limited experience with maralixibat, the safety of the product in the long term is unknown and of importance. Therefore, the applicant should consider including "long-term safety" as missing information in the list of safety concerns.

It should be noted that a few RMP sections requires amendments as follows:

Part II SI: The applicant should delete evaluation of efficacy of currently used off-label pharmaceutical therapeutics. **(OC)**

Part II SII: The applicant should focus on the safety findings and delete statement of mechanism of action. **(OC)**

Part II SIII: The applicant should clearly state what data was used for the safety assessment. With regard to the limited number of patients on long-term exposure and considering that studies are ongoing, the applicant should update the exposure data if information from a later data cut off is available. **(OC)**

Part II SIV: The applicant should clarify if just data in the elderly or data in adults are missing and address this accordingly. As it cannot be excluded that patients will use lipid binding resins concomitantly to maralixibat and this concomitant treatment may cause/aggravate malabsorption of concomitantly administered lipophilic medications or lipophilic vitamins, "malabsorption of fat-soluble drugs, food components and vitamins" should considered to be included as important potential risk. **(OC)**

Part II SIV: Patients weighing above 50 kg were excluded from clinical trials. The applicant stated that no notable change of sBA was noticed in obese patients. The applicant should comment if body weight over 50 kg affects maralixibat efficacy and if so how this issue will be addressed as a lack of efficacy in severely ill patients is also a risk. **(OC)**

3.4.3. Conclusions on the safety specification

Having considered the data in the safety specification, the CHMP considers that the following issues should be addressed:

Depending on the applicant´s response within the clinical safety part the applicant should consider including "Hepatotoxicity", "Clinically significant or severe diarrhoea leading to dehydration and electrolyte imbalance" and "malabsorption of fat-soluble drugs, food components and vitamins" as an important potential risk. Additionally, the applicant should consider including "long-term safety" as missing information. **(OC)**

3.4.4. Pharmacovigilance plan

Table Part III.1: On-going and planned additional pharmacovigilance activities

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 – Required additional pharmacovigilance activities				
None				

No additional PhV activities are proposed.

Overall conclusions on the PhV Plan

The PRAC opinion is that there is a need to collect notably further amount of data on long-term safety and efficacy of maralixibat in PFIC patients. Therefore, the Applicant is requested to explore the possibility of using available disease registries and discuss the feasibility of collaboration with the PFIC Network Patient Registry for a new Registry which could be included as an additional pharmacovigilance activity in the proposed RMP. **(OC)**

3.4.5. Risk minimisation measures

Routine risk minimisation measures

None.

Additional risk minimisation measures

No additional risk minimisation measures are proposed.

Overall conclusions on risk minimisation measures

The PRAC having considered the data submitted was of the opinion that:

The proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication. This section may be subject to further changes depending on the final list of safety concerns proposed by the CHMP.

3.4.6. Conclusion on the RMP

The CHMP and PRAC considered that the risk management plan version 0.1 is not acceptable. Details are provided in the endorsed Rapporteur assessment report.

3.5. Pharmacovigilance system

The Applicant/Proposed Future MAH has submitted a signed Summary of the Applicant's/Proposed Future MAH's Pharmacovigilance System. Provided that the Pharmacovigilance System Master File fully complies with the new legal requirements as set out in the Commission Implementing Regulation and as detailed in the GVP module, the RMS/CHMP considers the Summary acceptable.

Periodic Safety Update Reports submission requirements

4. Significance and Conformity of paediatric studies

Significance of paediatric studies

N/A

Conformity with agreed Paediatric Investigation Plan

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0200/2020 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0200/2020 was not yet completed as some measures were deferred.

5. Benefit risk assessment

5.1. Therapeutic Context

5.1.1. Disease or condition

Maralixibat is intended for *treatment of Progressive Familial Intrahepatic Cholestasis (PFIC) Type 2 in patients 1 year of age and older*.

PFIC2 is the most common subtype of PFIC (50%–60% of all patients with PFIC) and is caused by a mutation at ABCB11 leading to varying degrees of deficiency in the bile salt export pump (BSEP) and consequently impairment in bile acid excretion capabilities with cholestasis. Three categories of BSEP dysfunction have been described with BSEP 1 and BSEP2 representing mild and moderate levels of dysfunction (so called nt-PFIC2) and BSEP3 representing the most severe subtype of t-PFIC2 with complete loss of BSEP function.

PFIC2 typically manifests in young age. Affected children suffer from intractable pruritus, disturbed sleep, reduced quality of life, fatigue, fat malabsorption, deficiencies in fat-soluble vitamins, and growth retardation. Patients with PFIC2 are at increased risk to develop hepatic impairment or hepatocellular carcinoma (HCC) at an early age and have limited life expectancy. The only definitive treatment available thus far is liver transplantation.

5.1.2. Available therapies and unmet medical need

Current pharmacotherapeutic options for treatment of PFIC2 include off-label use of ursodeoxycholic acid (UDCA), cholestyramine, and rifampicin. UDCA, is used to promote biliary flow by increasing the hepatocyte excretion of bile acids and inhibiting intestinal reabsorption (Mehl et al. 2016), and has been reported to improve pruritus in some patients. Oral bile acid binding resin, cholestyramine, prevents bile acids from entering the enterohepatic cycle, and rifampicin, upregulates hepatic excretion of bile salts. However, both drugs have only limited or temporary efficacy, and rifampicin can cause hepatotoxicity. Further options to relieve pruritus include antihistamines, steroids, and naltrexone (Düll and Kremer 2020). However, these also display weak or limited efficacy. Overall, the existing pharmacological therapies often fail, and surgical treatment, i.e., SBD and liver transplantation, commonly becomes necessary. Approximately 50% of the patients benefit from SBD and almost all from liver transplantation. However, surgical procedures are associated with short- and long-term surgical and medical complications as well as a significant patient and caregiver burden, e.g., from a lifelong stoma and the need for repeat surgeries.

In patients with PFIC2, only around 45% and 32% will survive with their native liver to Year 10 and Year 18, respectively (van Wessel et al. 2020).

There is a high unmet medical need in treatment of PFIC2.

Maralixibat is a substance with a novel substance that inhibits apical sodium dependent bile acid transporter (ASBT) in the terminal ileum, thus interrupting the enterohepatic circulation of bile acids and reducing systemic bile acid levels. Maralixibat may thus, at least partly, address this unmet medical need.

5.1.3. Main clinical study

Key evidence of efficacy and safety in the targeted indication is provided from one small Phase 2 exploratory, non-randomised, single-arm long-term treatment study (LUM001-501) that included 25 patients with PFIC2 in the age range of 1 to 13 years (median age 4 years). Patients with non-truncating (19 participants) and truncating (6 participants) forms of PFIC2, increased levels of sBA (>3xULN), without liver cirrhosis, hepatic tumour, or prior surgical treatment were studied. Of note, genetically confirmed diagnosis could be located only for 12 of 25 patients. The study had titration (8 weeks), maintenance (Week 8 to Week 13), extension (Week 13 to Week 76) and follow-up phases (from Week 76 to end of study) and patients were treated for up-to more than 5 years.

Main objective was to evaluate short and long-term safety and efficacy of maralixibat. Short-term efficacy was assessed at Week 13 of the treatment by means of changes from baseline in mean fasting sBA (primary endpoint), ALT, bilirubin (total and direct), pruritus as measured by ItchRO (Observer ItchRO/patient ItchRO; 4-weeks average score) (secondary endpoints). Long-term efficacy was assessed by evaluating the same parameters and changes in z-scores for growth, quality of life, fatigue, and biochemical markers of cholestasis. Various responder analyses and transplant-free analysis in responder vs. non-responder population were also assessed.

Additional supportive data are derived from a retrospective comparison of event free survival (EFS) (events defined as hard clinical endpoints, i.e., liver transplantation, surgery of biliary diversion (SBD), or death) in responder (sBA of < 102 µmol/L or reduced by ≥ 75%) and non-responder patients from the main study and the external natural history control group from the NAPPED registry (NAtural Course and Prognosis of PFIC and Effect of Biliary Diversion Study Group). NAPPED registry is a non-interventional, multi-national, multi-center longitudinal registry of approximately 700 PFIC patients. The NAPPED consortium included 48 tertiary referral centers around the world at the time the retrospective analysis was conducted.

5.2. Favourable effects

On short-term treatment, there were no favourable effects detected as per the primary analysis. Among the evaluated secondary endpoints, numerical and statistically significant improvement was observed only for one parameter, i.e., the mean (SD) ItchRO(Obs) 4-week average morning score (-0.715 (0.1179) (95% CI: -0.959, -0.472; p<0.0001)).

On long-term treatment mean values of the majority of efficacy parameters showed improvement in the extension phase. Specifically, favourable and clinically relevant improvements were observed in sBA (sBA of < 102 µmol/L or decreased by ≥ 75% compared to baseline) and pruritus (Mean change (SD) of -1.970 (0.2925) points in the average weekly morning score of ItchRO(Obs)) in the responder patients (6/25 patients with PFIC2), which were accompanied by improvements in quality of life (mean and median scores) and fatigue (median scores). Improvements in z-scores for height and weight were also observed in the subgroup of responders.

Decrease in sBA in the responder population was observed already at weeks 2-4 of treatment and was maintained till end of the study.

Comparison of TFS between responders and non-responders treated with maralixibat showed that 100% of study participants who achieved the sBA response criteria remained transplant free, whereas the majority of participants without sBA response went on to receive a liver transplant. The difference between the groups was statistically significant (p=0.0006), suggesting potential favourable effects of maralixibat treatment on TFS.

Overall, the reported favourable effects were consistent in the responder population, apart from the cases when parameters like ALT, AST and bilirubin showed no improvements in the patients with normal/close to normal baseline values.

5.3. Uncertainties and limitations about favourable effects

The main study was a small open-label uncontrolled study that was not planned as a confirmatory study. High number of drop-outs was observed and changes in use of concomitant medication were reported. Small sample size, absence of blinding, absence of a control group, variable use of concomitant treatment and variable number of patients treated create major uncertainties.

Formally, the study is a failed study as the main analysis (i.e. primary and secondary endpoints) did not show efficacy of maralixibat. Key data presenting possible favourable effects of maralixibat have been derived from a subgroup of responders and post hoc (transplant-free analysis between responder and non-responder and event free analyses against historical data) analyses only. In addition, these analyses were planned and conducted in knowledge of data, and are burdened with multiple confounders (e.g. baseline differences between responders and non-responders, e.g. responders had less severe disease compared to non-responders, between the study and the registry populations), which could have potentially biased the results.

Additional potential confounding is related to the types of the parameters chosen and selected baseline variables. Subjective parameters, such as quality of life and pruritus assessments are susceptible to bias and detection of true treatment effects in an open-label non-controlled setting is challenging. Objective parameters, such as sBA, ALT, may undergo natural fluctuations and single values (as was the case in the main study) may not be suitable to adequately characterise true baseline status of the respective parameters, or the disease.

A disproportional distribution of responders was noted across study centers and major (and potentially also minor) protocol deviations were noted, which raise concerns on GCP-conformity and, overall, integrity of the study data.

Additional uncertainties are created by the fact, that it is currently unclear whether all patients included had confirmed diagnosis of PFIC2. Moreover, the study population, and especially the responder patients, are not considered representative of general PFIC2 population and generalisability of efficacy data to broader PFIC2 population is unclear. Additionally, no favourable effects were observed in any of the patients with t-PFIC2 type of disease and there are no data available in the population older than 14 years of age. Consequently, there is an uncertainty whether the study data can be extrapolated to the targeted broad indication (patients with PFIC2 1 year old and older).

Dedicated dose-finding study was not conducted in the targeted population and dosing recommendations need to be further discussed, particularly the starting and maximum doses.

The presented comparative TFS and EFS analyses conducted between maralixibat-responders vs. non-responders and between the study population and historical controls, respectively, are burdened with multiple methodological deficiencies, so that there is an uncertainty whether and to which extent the observed positive effects can be ascribed to maralixibat.

5.4. Unfavourable effects

Safety data in PFIC are limited to 33 patients due to the rare orphan disease nature of PFIC. The applicant has tried to overcome this limitation by submitting additional safety data from 86 children with Alagille-Watson-Syndrom (ALGS), another cholestatic liver disease in the paediatric population.

Based on the submitted safety data, the drug seems to add a mild to moderate toxicity to a PFIC as well as ALGS mixed- population treated.

This toxicity is described by the following key safety characteristics in the PFIC target population: All children (33/33) had at least 1 TEAE and treatment-emergent AEs potentially related to study drug occurred in 26/33 PFIC-participants (78.8%). SAEs were observed in 24/33 (45.5%) subjects, 5/24 (15.2%) of the SAEs were presumed to be potentially related to maralixibat. Discontinuation rate due to TEAE was high and occurred in 10/33 children (30.3%).

In accordance with the known safety profile of the pharmacological class of ASBT inhibitors, GI events including diarrhoea (57.6%) and abdominal pain (45.5%) were the most frequently reported adverse drug reactions for maralixibat for the applied PFIC target-population included in study LUM001-501. The majority of these events were described to be mild to moderate in severity, transient in nature, and resolved with no action taken with maralixibat and no special approaches for monitoring were required. Safety data in the other paediatric population of ALGS subjects from the 13-week, placebo-controlled studies (LUM001-301 and LUM001-302) revealed a similar incidence of events of diarrhoea in the overall maralixibat and placebo groups (43.6% vs. 44.4% of participants, respectively), while events of abdominal pain were slightly rarer reported in M: 25.6% versus P: 16.7%, respectively.

Specific in the PFIC-population, the most frequent treatment-related TEAEs were similar frequent: diarrhoea (13 participants [39.4%]), abdominal pain (9 participants [27.3%]), abdominal pain upper (6 participants [18.2%]), vomiting (5 participants [15.2%]), and INR increased (5 participants [15.2%]). In 6.1% of the subjects (n=2/33) hyperbilirubinaemia was observed. The frequencies and TEAEs are in line the most common TEAEs (> 10% overall) in every study (including long term exposure) in the ALGS population, which are reported as abdominal pain (45,5%), cough (34.5), diarrhea (39.4%), headache (20.7%), nasopharyngitis (34.5%), pyrexia (48.3), upper respiratory tract infections (20.7%) and vomiting (37.9%). (e.g. from LUM001-304). Median time to first onset for

events of diarrhoea and abdominal pain was 30 days and 61 days, respectively. The duration of the gastrointestinal events ids described as short with a median duration for events of diarrhoea and abdominal pain of 2 days and 1 day, respectively.

In PFIC population included in Study LUM001-501 about half of the participants (45.5%) had an SAE. Diarrhea, abdominal pain, and gastroenteritis were the only SAEs (based on PT) that were reported in more than 1 participant (each SAE was reported in 2 participants; 6.1%). 5 of these SAEs (15.2%, all in 280µg/kg QD) were classified as potentially drug-related. The incidence of SAEs in the ALGS open-label, long-term extension studies is reported slightly lower with about 29.8% (25/84 participants) for participants exposed to maralixibat. The available limited placebo comparison from LUM001-301/302) seems not to indicate a significant difference regarding SAEs between maralixibat and placebo. No child died during the trials.

Laboratory data showed several probably isolated, asymptomatic significant elevations in ALT in some ALGS participants; while no event is described in the PFIC patients. Although the underlying liver disease might have contributed to this finding, the DSM was sufficiently concerned and triggered an assessment of these events by an independent panel of liver experts. The experts concluded that based on the literature, the accumulated pre-clinical and clinical trial data as well as the detailed independent external liver safety review, there is evidence that maralixibat may cause ALT elevations in a certain percentage (2-5% probably, 10-20% probably or possibly related events) of subjects with ALGS. No predictors of this treatment response have been identified so far. None of the observed events were assessed as serious and none led to liver-related morbidity or mortality.

Relevance of some changes in the cholestasis parameters (e.g. FGF19) may be suggestive of potential risk of increased bile acid synthesis. This issue needs to be further discussed and clarified.

A total of 10 participants (30.3%) had an AE that resulted in discontinuation of maralixibat in trial LUM001-501. 3 of the 10 (9.1%) had an event considered possibly related to maralixibat, and 7 (21.2%) had an event considered as unlikely or not related to maralixibat. However, events of blood bilirubin increased (4 participants [12.1%]) and disease progression (2 participants [6.1%]) were the main reasons for treatment discontinuation in this trial.

5.5. Uncertainties and limitations about unfavourable effects

Safety reporting in this applicant is largely insufficient and concerning. This is only partially explained by the limited number of patients available in a rare paediatric orphan disease population. Since a comparative phase 3 trial in the PFIC population remains missing, a high degree of uncertainty burden an adequate assessment in general. Additional clarification is needed to understand the currently fragmentary information provided. Insofar, the current safety assessment has to be seen as preliminary.

The safety population is not only too small to identify reliably signals for infrequent or rare toxicities, but it remains also open whether the PFIC subtype 1 and especially the ALGS subjects could be included for safety assessment as proposed. This needs justification.

The safety assessment is hampered by the fact that the observed safety event may reflect the underlying disease as well as drug-related TEAEs. Assessment of drug-relationship of TEAEs in not always comprehensible and the selected approach for causality assessment needs to be justified.

Safety assessment in the applied target population of PFIC2 children is based on the results from 25 subjects suffering from PFIC 2 and additional 8 children with the not applied PFIC 1 subtype in the pivotal trial LUM001-501. However, safety data from a larger phase 2 population of 86 children suffering from ALGS and also treated with maralixibat in 4 trials (LUM001-304, 301/302 and 305) was

additionally submitted. Of note, in ALGS patients, higher doses were administered than in PFIC2 patients. However, impact of potential differences needs to be discussed, considering that hepatotoxicity adverse events were identified significantly more frequent in the ALGS in the context of the maralixibat treatment.

Placebo controlled safety data is only available for a small ALGS population for a short term of several weeks. More mature data from longer placebo-controlled treatment would be needed to discriminate drug-related adverse events, this data is deemed too limited to allow a reliable comparison and identification of signals.

Differences in the posologies in the PFIC and ALGS trials are obvious, but not adequately discussed. Similarly, information on drug exposure in the trials is insufficiently and not comprehensible presented. Thus, the assessment of relevant details of exposure, impact of dose modifications/escalation strategies as well as regarding the degree of toxicities remains missing.

Although the product was investigated in a significantly larger adult population (~ 1600 patients !) suffering also from cholestatic liver diseases [Primary Biliary Cholangitis (PBC), Primary Sclerosing Cholangitis (PSC)] as well as hypercholesterolemia, this source of information was not prepared and submitted. In particular, for the clarification of the potential intrinsic hepatotoxicity and the causality of maralixibat for the observed toxicities in the paediatric trials this data could be valuable. At least a summary of analyses should be presented in order to identify potential "Hy's law " cases and other signals for more rare events.

Although the external hepatic safety panel concluded that there is currently no evidence that maralixibat causes bilirubin elevations in patients with ALGS and there is insufficient evidence of an association between bilirubin or liver enzyme elevations and maralixibat treatment in patients with PFIC, the long-term consequences of the observed ALT elevations remain unclear. Since the overall approach of reporting the hepatic safety data is not acceptable, additional clarification and comprehensible preparation of the liver safety data is requested. Moreover, the data in adults need to be reviewed and analysed to further conclude regarding this potentially most relevant identified risk in the paediatric population.

Maralixibat was designed to be minimally absorbed following oral administration because the site of action is within the lumen of the GI tract. It is metabolically stable in vivo and excreted almost exclusively in the feces as intact parent drug. However, it needs to be considered from the non-clinical data that in the human paediatric population bioavailablity may increase. A more permeable intestinal barrier may result in situations in which the gastrointestinal system is chronically disturbed and could lead to higher blood levels of maralixibat and induce other systemic adverse events than in children with the expected low plasma levels. It seems possible that the rather unspecific symptoms like abdominal pain, diarrhea or vomiting are associated with a higher permeable intestinal barrier. The association between the presence of such TEAEs and development of elevated transaminases should be analysed and discussed.

Subgroup analyses regarding the impact of body weight on safety events and others remains missing and needs to be submitted since this important in a paediatric population, which applies for use in children > 1 year of age.

There is a lack of information regarding potential immunological events and the allergenic potential of maralixibat (including the risk for topical immunisation). Since symptoms as abdominal pain and diarrhoea may be also caused by this class of events further clarification and discussion is needed.

The impact of maralixibat on absorption of FSV and the approach, how this adverse event was studied systematically in the clinical studies is not adequately evaluable. This needs further clarification.

Specific mitigation activities for the management of GI symptoms, including diarrhea and abdominal pain, during the clinical trials were given in the study protocols. However, it seems that these recommendation are not fully reflected in the currently proposed product information. This needs to be done. Similarly the applicant needs to explain and justify these proposals are also appropriate to be used in younger children.

5.6. Effects Table

Table 15 Effects Table for Livmarli in treatment of PFIC2 (data cut-off: 08 May 2020).

Effect	Short Description	Unit	Maralixibat	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
CFB in sBA at Week 13/ET **(primary endpoint)**	Change from baseline to Week 13/ET in mean fasting sBA levels	Mean (95% CI)	-38 (-114.598, 39.082)	N/A Comparison to baseline	Absence of control and blinding; small sample size; Single value chosen as a baseline; Possible confounding due to concomitant treatments/ Objective parameter that is less susceptible to placebo effects. Weak evidence	LUM001-501
Secondary endpoints						
CFB in ALT at Week 13/ET	Change from baseline to Week 13/ET in mean values	Mean (95% CI)	-11.3 (-41.6, 19.0)	N/A Comparison to baseline	Absence of control and blinding; small sample size; Single value chosen as a baseline; only mildly increased in part of the patients/ Objective parameter that is less susceptible to placebo effects. Weak evidence	LUM001-501
CFB in total bilirubin at Week 13/ET	Change from baseline to Week 13/ET in mean values	Mean (95% CI)	-0.02 (-0.41, 0.37)	N/A Comparison to baseline	Absence of control and blinding; Single value chosen as a baseline; normal at baseline in part of patients/ Objective parameter that is less susceptible to placebo effects. Weak evidence	LUM001-501
CFB in direct bilirubin at Week 13/ET	Change from baseline to Week 13/ET in mean values	Mean (95% CI)	-0.026 (-0.335, 0.283)	N/A Comparison to baseline	Absence of control and blinding; Single value chosen as a baseline; normal at baseline in part of patients/ Objective parameter that is less susceptible to placebo effects. Weak evidence	LUM001-501

Effect	Short Description	Unit	Maralixibat	Control	Uncertainties/ Strength of evidence	References
CFB in ItchRO(Obs) 4-Weeks average morning score 13/ET	Change from baseline to Week 13/ET in mean values	Mean (95% CI)	-0.697 (-0.954, -0.441)	N/A Comparison to baseline	Absence of control and blinding; Possible confounding due to concomitant treatments; subjective parameter susceptible to placebo effects/ "statistically significant" effects. Weak evidence	LUM001-501
CFB in ItchRO(Pt) 4-Weeks average morning score 13/ET	Change from baseline to Week 13/ET in mean values	Mean (95% CI)	-0.705 (-1.234, -0.175)	N/A Comparison to baseline	Absence of control and blinding; Possible confounding due to concomitant treatments; subjective parameter susceptible to placebo effects; only 7 patients analysed Weak evidence	LUM001-501
sBA responder rate*	Decrease in sBA of < 102 µmol/L or by ⩾ 75% compared to baseline	% (n/N)	28% (7/28)	N/A Comparison to baseline	Absence of control and blinding; small sample size; Single value chosen as a baseline; Possible confounding due to concomitant treatments/ Objective parameter that is less susceptible to placebo effects. Stable and sustained decrease in sBA levels over months and years. Weak evidence	LUM001-501
CFB in ItchRO(Obs) weekly morning average score	Mean change from baseline in ItchRO(Obs) weekly average scores	Points Mean (SD)	-1.970 (0.2925)	N/A Comparison to baseline	Absence of control and blinding; small sample size; Possible confounding due to concomitant treatments/ Sustained decrease. Weak evidence	LUM001-501
CFB in z-scores for height – resp. vs. non-responder	Change in the mean scores of z-scores for height in responders vs. non-responders	-	Slight improvement in z-scores in the responder subgroup; no improvement/slight worsening in non-responders	N/A Responder vs. non-responder	Absence of randomisation; small sample size; differences in baseline characteristics of the subgroups/weak evidence	LUM001-501
TFS sBA resp* vs. non-resp (PFIC2)	Transplant-free survival in sBA responder vs. non-responder	-	Responders 100% transplant free (p=0.0018)	Responder vs. non-responder	Absence of randomisation; differences in baseline characteristics of the subgroups/weak evidence	LUM001-501
Unfavourable Effects						

Effect	Short Description	Unit	Maralixibat	Control	Uncertainties/ Strength of evidence	References
TEAE		n/N %	33/33 100%	N/A	Uncontrolled data from LUM001-501	LUM001-501
Drug-related TEAE	TEAE Potentially Related to Study Drug	n/N %	26/33 78.8%	N/A	Uncontrolled data from LUM001-501	LUM001-501
Serious TEAE		n/N %	15/33 45.5%	N/A	Uncontrolled data from LUM001-501 (PFIC)	LUM001-501
Drug related SAE	Serious TEAE Potentially Related to Study Drug	n/N %	5/33 15.2%	N/A	Uncontrolled data from LUM001-501 (PFIC)	LUM001-501
Death	Death	n/N %	0	N/A	Uncontrolled data from LUM001-501 (PFIC)	LUM001-501
Drug related Discontinuation	TEAE Leading to Study Drug Discontinuation	n/N %	10/33 30.3%	N/A	Uncontrolled data from LUM001-501 (PFIC)	LUM001-501
Diarrhoea		n/N %	19/33 57.6 %	N/A	Uncontrolled data from LUM001-501 (PFIC)	LUM001-501
Abdominal pain	PT= Abdominal+ upper abdominal pain	n/N %	23/33 75.5%	N/A	Uncontrolled data from LUM001-501 (PFIC)	LUM001-501
Vomiting		n/N %	17/33 51.5 %	N/A	Uncontrolled data from LUM001-501 (PFIC)	LUM001-501

Abbreviations: sBA – serum bile acid; ET – end of trial; CFB – change from baseline; TFS – transplant-free survival, EFS – event-free survival; MRS - maralixibat
Notes:* Responder defined as sBA < 102 µm/L or a 75% reduction in sBA compared with baseline

5.7. Benefit-risk assessment and discussion

5.7.1. Importance of favourable and unfavourable effects

Primary and secondary endpoints of the main study, reduction in sBAs, ALT, bilirubin and pruritus, after 13 weeks of treatment with maralixibat did not show significant treatment effects in overall PFIC2 population. Favourable effects on the same parameters were, however, observed in a sub-population of the patients with PFIC2 (about one quarter of the patients). These effects developed within 2-4 weeks of start of the treatment in this subgroup of patients and seemed to remain constant over the months and years on treatment, suggesting maintenance of effect.

In the patients with PFIC2 increased levels of sBA occupy a key role in the disease progression/liver damage and clinical symptomatology (e.g., by causing pruritus). Decreased levels of sBA are, therefore, expected to translate into relevant clinical benefits, such as improvement in pruritus, slower progression of the disease and delay in need of surgical treatment (for reasons of pruritus, hepatic cancer, or failure). Indeed, study data suggest that the patients with decreased levels of sBA also experienced improvements in pruritus, quality of life and reduced fatigue. These favourable effects are considered clinically relevant, provided that these are proven to be attributable to the treatment with maralixibat. Further, analysis of transplant-free survival in the patients included in the study and the comparisons of event-free survival (events defined as surgical biliary diversion, liver transplantation, or

death) between the study patients and external historical control suggest that maralixibat may have favourable effects on timing of the above events.

Another potential effect of maralixibat may be positive impact on growth. Since growth retardation is one of the disturbing and burdening symptoms of PFIC2, improvement in z-scores of growth and weight, which was observed in the maralixibat responders is considered meaningful.

Overall, to summarize, a number of relevant favourable effects were observed in the patients, who had clinically significant decrease in sBA levels in response to maralixibat. Whether or not these effects can be attributed to the administered treatment, and whether these indeed translate into relevant clinical benefits, is currently unclear and remains to be proven. Favourable results in a subgroup of patients from an overall failed study may be considered hypothesis-generating but require confirmation.

Currently, an adequate assessment of safety profile is significantly hampered due to many remaining uncertainties and by a significant amount of missing or unclear information in the submitted documents and analyses. Moreover, the fact that the observed safety event may reflect the underlying disease as well as drug-related TEAEs, makes evaluation of causal relationship of reported adverse event TEAEs additionally difficult, in particular, since the general approach for identification of causality was not adequately transparent.

Since the PFIC 2 trial population available is very restricted, the applicant has enhanced the safety database by adding data from maralixibat exposed ALGS subject. However, the approach is neither justified nor were underlying differences between the populations addressed. Discrepancies in safety outcome between both populations may reflect disease intrinsic reasons, but cannot be evaluated. Thus, the reliability of the provided safety needs to be established. Currently the existing concerns regarding the quality of the safety data are so pronounced that only a preliminary risk assessment is possible.

Provided that sufficient clarification on the many open issues can resolve the reliability concerns and confirm the applicants view on the safety risks, the drug may add only a mild to moderate gastrointestinal toxicity to the children. This is reflected by abdominal pain, diarrhoea and vomiting. However, since assessment has revealed some suspects regarding a potential hepatotoxicity the question whether maralixibat's risk are fully evident at present cannot be answered and affirmed.

5.7.2. Balance of benefits and risks

The submitted clinical program is considered insufficient to support the targeted indication. The conducted phase 2 study in the PFIC2 population is not adequate. Neither are the collected data convincing and sufficient given numerous important uncertainties. Additional comparative analysis of study data with historical control cannot provide confirmative evidence for efficacy of maralixibat. Thus, firm conclusions on efficacy of maralixibat in the targeted patient population cannot be drawn based on the available data. Additional data are required in order to be able to draw final conclusions.

Treatment effects have not been established due to major uncertainties and benefits do not outweigh the seemingly mild AE profile of maralixibat.

5.7.3. Additional considerations on the benefit-risk balance

N/A

5.8. Conclusions

The overall B/R of Livmarli is negative.

6. Biosimilarity assessment

N/A

6.1. Pharmacovigilance system

None

6.2. New active substance status

None

7. Recommended conditions for marketing authorisation and product information in case of a positive benefit risk assessment

7.1. Conditions for the marketing authorisation

It is premature to decide on conditions for the marketing authorisation.

7.2. Summary of product characteristics (SmPC)

Non-clinical comment:

The wording of SmPC section 5.3 as proposed does not fully reflect the submitted data. The appropriate wording will depend on the responses of the Applicant given to the Other Concerns raised.

Clinical comments:

Please refer to the annotated PI.

7.3. Labelling

7.4. Package leaflet (PL)

The PL should be adapted to the changes in the SmPC.

User consultation

Preliminary issues/comments are included in the PI. All comments included in the SmPC only are also relevant to the PL as far as applicable.

8. Appendices (as appropriate)

N/A

QRD checklist for the review of user testing results

The applicant did not submit results of a user testing according to Article 59 (3)/61 (1) of Directive 2001/83 EC. Instead, the applicant submitted the following commitment:

Mirum Pharmaceuticals, Inc. commits to providing the results from the consultation with target patient groups (also called user tests) on the package leaflet and instructions for use together with the Day 121 responses.

CHMP comment:

The approach of the applicant can be accepted. Submission of a user testing will be required for the Day 121 responses and be included as other concern in the List of Questions