STATISTICAL ANALYSIS PLAN

Study Title:	Open-Label, Phase 2 Study to Evaluate the Safety and Tolerability of Maralixibat in the Treatment of Infants with Cholestatic Liver Diseases Including Progressive Familial Intrahepatic Cholestasis and Alagille Syndrome (RISE)
Name of Test Drug:	Maralixibat
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CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

7αC4	7α hydroxyl-4-cholesten-3-one
AE	adverse event
AESI	adverse event of special interest
ALGS	Alagille syndrome
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BID	twice daily
BMI	body mass index
CI	confidence interval
CSR	clinical study report
CSS	Clinician Scratch Scale
CTCAE	Common Terminology Criteria for Adverse Events
ECG	electrocardiogram
eCRF	electronic case report form
HLGT	high-level group term
HLT	high-level term
ID	identification
ItchRO	Itch-Reported Outcome
LLT	lower-level term
LOQ	limit of quantitation
LSV	lipid-soluble vitamin
LTE	long-term extension
MedDRA	Medical Dictionary for Regulatory Activities
MRX	maralixibat
Obs	Observer
PFIC	progressive familial interhepatic cholestasis
PK	Pharmacokinetic
PRO	patient-reported outcome
PT	preferred term
Q1, Q3	first quartile, third quartile
QD	once daily
QoL	quality of life

SAE	serious adverse event
SAP	statistical analysis plan
sBA	serum bile acid
SD	standard deviation
SE	standard error
SOC	system organ class
TEAE	treatment-emergent adverse event
TFLs	tables, figures, and listings
TSB	total serum bilirubin
ULN	upper limit of normal
WHO	World Health Organization
WHODrug	WHO Drug Dictionary

1. INTRODUCTION

This statistical analysis plan (SAP) describes the statistical analysis methods and data presentations to be used in tables, figures, and listings (TFLs) for the interim and final analyses to support the clinical study report (CSR) for Study MRX-801. This SAP is based on the study protocol Version 5, 14 January 2022, and the electronic case report form (eCRF). The SAP will be finalized before finalizing the data for the first interim analysis.

Any deviations from the protocol-specified analyses will be documented in Section 5.9.

1.1. Study Objectives

The aim of this study is to assess the safety and tolerability of maralixibat in infants <12 months of age with cholestatic liver disease due to ALGS or PFIC.

Maralixibat has been shown to be safe and well tolerated in >1600 exposed participants overall, including >150 children ≥12 months of age with cholestatic liver disease. Safety in children <12 months of age had not been assessed. Therefore, Study MRX-801 is assessing the safety and tolerability of maralixibat in infants <12 months of age.

1.1.1. Primary Objective and Endpoint

- To evaluate the safety and tolerability of maralixibat in infant participants with ALGS or PFIC:
    - Safety (incidence of treatment-emergent adverse events [TEAEs], including those that are serious, are related to maralixibat, that lead to withdrawal, are of special interest, along with TEAEs by severity and change from baseline in safety laboratory [including measurement of osmolality, osmolar gap, and anion gap when clinically indicated] and physical examination findings, vital signs, and neurodevelopmental assessment) and tolerability

1.1.2. Secondary Objectives and Endpoints

- To evaluate the treatment effect of maralixibat on sBA levels:
    - Change from baseline to Week 13 in fasting sBA levels
- To evaluate the effect on liver enzymes (ALT, AST) and bilirubin:
    - Change from baseline to Week 13 in liver enzymes (serum ALT, AST) and total serum bilirubin (TSB)

- To evaluate the effect on lipid-soluble vitamins (LSVs):
  - Change from baseline to Week 13 in vitamins A, D, E, and K. Changes in INR will also be assessed as a surrogate marker for vitamin K deficiency
- To evaluate the pharmacokinetics of maralixibat in infant participants:
  - Systemic maralixibat concentrations in plasma before dosing and 2.5 hours after the morning dose at specified time points

1.1.3. Exploratory Objectives and Endpoints

- To evaluate the impact of maralixibat on pruritus in study participants with pruritus at baseline:
  - Change from baseline in morning Itch Reported Outcome Observer (ItchRO[Obs]) instrument severity score
  - Change from baseline in evening ItchRO(Obs) severity score
  - Change from baseline in daily ItchRO(Obs) severity score (defined as maximum of the morning and evening score on a given day)
  - Change from baseline in Clinician Scratch Scale (CSS) score
- To evaluate the effect of maralixibat on growth:
  - Changes from baseline in height and weight and in mid-upper-arm and head circumferences (z-scores)
- To evaluate the impact of maralixibat on healthcare resource utilization:
  - Number of hospitalizations; emergency ward visits; and the length of stay for hospitalization, surgeries, and procedures related to the participant's disease type
- To evaluate the impact of maralixibat on caregiver burden:
  - Number of days the caregiver misses work

1.2. Study Design

This is an open-label, multicenter, Phase 2 study to evaluate the safety and tolerability of maralixibat in the treatment of infants (<12 months of age) with cholestatic liver disease (PFIC or ALGS). At least 6 participants will be enrolled into each cohort. The study periods are as follows:

1. Screening (up to 4 weeks)
2. Core Study Period
   a. Dose escalation (Weeks 1 to 6)
   b. Stable dosing (Weeks 7 to 13)
3. Long-Term Extension (LTE)

During the treatment period, participants receive standard-of-care treatment in line with permitted concomitant medications and with investigator and caregiver preference in addition to maralixibat.

Study cohorts are defined as:

- Participants with PFIC
- Participants with ALGS

In this study, a screening period of up to 4 weeks will be followed by 13 weeks of dose escalation and stable dosing. The total Core Study Period can last up to 17 weeks.

The Dose Escalation Period can range from 2 to 6 weeks. Stable dosing will occur at 400 µg/kg once daily (QD) for ALGS, and at 600 µg/kg twice daily (BID) for PFIC or at the highest tolerated dose. Dose escalation for maralixibat is described in detail in Section 6.2.3 of the protocol. For participants <1 month of age, the dose for both ALGS and PFIC is 75 µg/kg QD for the duration of treatment below this age boundary.

The investigator has up to Week 6 to escalate up to the target dose for ALGS and PFIC; if rechallenges or further dose escalations fail before Week 6, the participant will remain on the highest tolerated dose level for the Stable Dosing Period. Dose reductions are allowed for safety or tolerability reasons down to a minimum level of 200 µg/kg QD for ALGS and 150 µg/kg BID for PFIC. Participants who cannot tolerate this dose will be discontinued from the study.

At the end of the Core Study Period (Week 13), participants will continue into an LTE within Study MRX-801. Until the participant is ≥12 months of age, study clinic visits are scheduled every 4 weeks with a telephone contact between the visits. Once the participant is ≥12 months of age, study clinic visits are scheduled every 16 weeks. Participants will remain in the LTE until one of the following occurs:

- They are eligible to enter an expanded-access program or a named-patient program
- The drug is commercially available in the participant location
- The sponsor discontinues the program

1.3. Sample Size Determination

Participants who meet the study's inclusion and exclusion criteria will be enrolled in the study. The sample size is not based on statistical considerations. Investigations to date show maralixibat to be minimally absorbed (<1%); therefore, the sample size is not based on the assumption that 6 participants per cohort will allow for a meaningful determination of the PK profile in infants aged <12 months with cholestatic liver disease. In order to ensure an adequate representation of participant ages in the study, a minimum of 3 participants in each cohort must be <9 months of age at the baseline visit. Participants who discontinue from the Core Study Period will be replaced.

2. TYPE OF PLANNED ANALYSES

2.1. Interim Analyses

The sponsor will conduct interim analyses when at least 6 participants have completed the 13-week Core Study Period for each study cohort (ALGS and PFIC). Thereafter, the sponsor may also conduct interim analyses after 12 participants are enrolled and have completed the 13-week Core Study Period for each study cohort (ALGS and PFIC). This will have no impact on the final analysis.

The scope of the interim analyses will be a subset of all the analyses described in the SAP, depending on the needs of the sponsor. The interim analysis could be limited to a single cohort (ALGS or PFIC).

2.2. Final Analysis

A final analysis will be performed after all participants have completed the study.

3. GENERAL CONSIDERATIONS FOR DATA ANALYSES

Analysis results will be presented using descriptive statistics. For categorical variables, the number and percentage of participants in each category will be presented; for continuous variables, the number of participants (n), mean, standard deviation (SD), standard error of the mean (SE), median, first quartile (Q1), third quartile (Q3), minimum, and maximum will be presented.

By-participant listings will be presented for all participants in the Safety Analysis Set and sorted by disease (ALGS or PFIC), participant identification (ID) number, visit date, and time (if applicable). Data collected on log forms, such as AEs, will be presented in chronological order within each participant. Age, sex, race, and ethnicity will be included in the listings, as space permits.

3.1. Analysis Sets

The Safety Analysis Set is defined as all participants who enrolled and received at least 1 dose of maralixibat during Study MRX-801. All safety endpoints will be analyzed using the Safety Analysis Set.

The Efficacy Analysis Set is defined as all participants who enrolled. All efficacy endpoints will be analyzed using the Efficacy Analysis Set. The following endpoints are considered to be efficacy endpoints: sBA, ItchRO and CSS.

A treatment policy estimant will be used for all analyses using the Safety Analysis Set. For analyses using the Efficacy Analysis Set, a treatment policy estimand will be used with the exception of the intercurrent event of PEBD. If participants undergo PEBD, any data collected after PEBD will be excluded. Such an event is not expected to occur. However, such a procedure will likely confound the interpretation of the treatment effect of maralixibat.

A listing of reasons for exclusion from analysis sets will be provided by participant.

3.2. Participant Grouping

Not applicable for this study.

3.3. Strata and Covariates

Not applicable for this study.

3.4. Examination of Participant Subgroups

Analyses will be performed separately for the ALGS and PFIC cohorts. No other subgroups are planned.

3.5. Multiple Comparisons

No adjustment for multiplicity will be made.

3.6. Missing Data and Outliers

3.6.1. Missing Data

In general, missing data will not be imputed unless methods for handling missing data are specified. Exceptions are presented in this document.

For missing last dosing date of study drug, imputation rules are described in Section 4.6.1. The handling of missing or incomplete dates for prior and concomitant medications is described in Section 4.5 and for AE onset is described in Section 5.1.5.2.

3.6.2. Outliers

Outliers will be identified during the data management and data analysis process. Sensitivity analyses may be conducted to assess the robustness of the results.

3.7. Data Handling Conventions and Transformations

In general, age (in months) on the first dosing date of study drug will be used for analyses and presentation in listings. If an enrolled participant was not dosed with any study drug, the enrollment date will be used instead of the first dosing date of study drug. For screen failures, the date the informed consent form was signed will be used for age calculation. If only the birth year is collected on the eCRF, "01 July" will be used for the unknown birth day and month for the purpose of age calculation. If only birth year and month are collected, "01" will be used for the unknown birth day.

Data that are continuous in nature but are less than the lower limit of quantitation (LOQ) or above the upper LOQ will be imputed as follows:

- A value that is 1 unit less than the LOQ will be used to calculate descriptive statistics if the datum is reported in the form of "<x" (where x is considered the LOQ). For example, if the values are reported as <50 and <5.0, values of 49 and 4.9, respectively, will be used to calculate summary statistics. An exception to this rule is any value reported as <1 or <0.1, etc. For values reported as <1 or <0.1, a value of 0.9 or 0.09, respectively, will be used to calculate summary statistics.

- A value that is 1 unit above the LOQ will be used to calculate descriptive statistics if the datum is reported in the form of ">x" (where x is considered the LOQ). Values with decimal points will follow the same logic as above.

- The LOQ will be used to calculate descriptive statistics if the datum is reported in the form of "≤x" or "≥x" (where x is considered the LOQ).

If methods based on the assumption that the data are normally distributed are not adequate, analyses may be performed on transformed data or nonparametric analysis methods may be used, as appropriate.

3.8. Analysis Visit Windows

3.8.1. Definition of Study Day

Study day will be calculated from the first dosing date of study drug and derived as follows:

- For postdose study days: Assessment Date – First Dosing Date + 1
- For days prior to the first dose: Assessment Date – First Dosing Date

Therefore, Study Day 1 is the date of the first dose of study drug.

3.8.2. Analysis Visit Windows

Each protocol-specified vists allows a visit window for that visit; for the analysis, observations will be assigned to analysis visit windows as shown in Table 1.

Table 1 Data and Tables Describing Corresponding Analysis Visit Windows

Data	Table Describing Analysis Visit Windows
Physical examination and vital signs	Table 2
ItchRO(Obs), Chemistry Panel, Lipid Panel	Table 3
Mid-upper-arm and head circumferences and PK samples	Table 4
Neurodevelopmental assessment and 12-lead ECG	Table 5
Healthcare resource utilization	Table 6
Clinician Scratch Scale, CBC with differential, and Lipid soluble vitamins	Table 7
Coagulation, 7αC4, sBA	Table 8

Table 2 Analysis Visit Windows for Physical Examinations and Vital Signs

Nominal Visit	Nominal Study Day	Lower Limit	Upper Limit
Baseline	1	(none)	1
Week 1	8	2	11
Week 2	15	12	18
Week 3	22	19	25
Week 4	29	26	32
Week 5	36	33	39
Week 6	43	40	56
Week 10	71	57	84
Week 13	92	85	105
LTE Week 17	120	106	133
LTE Week K† (K is every 4 weeks after previous visit)	K*7+1	(K-2)*7+1	(K+2)*7
LTE Week L‡ (L is every 16 weeks after previous visit)	L*7+1	(L-8)*7+1	(L+8)*7

Note: † schedule for participants <12 months of age. ‡ schedule for participants ≥12 months of age.

Table 3 Analysis Visit Windows for ItchRO(Obs), Chemistry Panel, Lipid Panel

Nominal Visit	Nominal Study Day	Lower Limit	Upper Limit
Baseline	1	(none)	1
Week 3	22	2	31
Week 6	43	32	56
Week 10	71	57	84
Week 13	92	85	105
LTE Week 17	120	106	133
LTE Week K† (K is every 4 weeks after previous visit)	K*7+1	(K-2)*7+1	(K+2)*7
LTE Week L‡ (L is every 16 weeks after previous visit)	L*7+1	(L-8)*7+1	(L+8)*7

Note: † schedule for participants <12 months of age. ‡ schedule for participants ≥12 months of age; ItchRo(Obs) is not collected once participant is in this cycle.

Table 4 Analysis Visit Windows for Mid-Upper-Arm and Head Circumferences and PK Samples

Nominal Visit	Nominal Study Day	Lower Limit	Upper Limit
Baseline	1	(none)	1
Week 6	43	2	56
Week 10	71	57	84
Week 13	92	85	105
LTE Week 17	120	106	133
LTE Week K† (K is every 4 weeks after previous visit)	K*7+1	(K-2)*7+1	(K+2)*7
LTE Week L‡ (L is every 16 weeks after previous visit)	L*7+1	(L-8)*7+1	(L+8)*7

Note: PK Samples not collected after Week 13. † schedule for participants <12 months of age. ‡ schedule for participants ≥12 months of age.

Table 5 Analysis Visit Windows for Neurodevelopmental Assessment and 12-Lead ECG

Nominal Visit	Nominal Study Day	Lower Limit	Upper Limit
Baseline	1	(none)	1
Week 13	92	2	105
LTE Week L‡ (L is every 16 weeks after previous visit)	L*7+1	(L-8)*7+1	(L+8)*7

Note: 12-lead ECG not performed in the LTE phase. ‡ schedule for participants ≥12 months of age. Neurodevelopmental assessment needs to be performed only once a year.

Table 6 Analysis Visit Windows for Healthcare Resource Utilization

Nominal Visit	Nominal Study Day	Lower Limit	Upper Limit
Baseline	1	(none)	1
Week 13	92	2	105
LTE Week 17	120	106	133
LTE Week K† (K is every 4 weeks after previous visit)	K*7+1	(K-2)*7+1	(K+2)*7
LTE Week L‡ (L is every 16 weeks after previous visit)	L*7+1	(L-8)*7+1	(L+8)*7

Note: † schedule for participants <12 months of age. ‡ schedule for participants ≥12 months of age.

Table 7 Analysis Visit Windows for Clinician Scratch Scale, CBC with Differential, and Lipid-Soluble Vitamins

Nominal Visit	Nominal Study Day	Lower Limit	Upper Limit
Baseline	1	(none)	1
Week 6	43	2	67
Week 13	92	68	105
LTE Week 17	120	106	133
LTE Week K† (K is every 4 weeks after previous visit)	K*7+1	(K-2)*7+1	(K+2)*7
LTE Week L‡ (L is every 16 weeks after previous visit)	L*7+1	(L-8)*7+1	(L+8)*7

Note: † schedule for participants <12 months of age. ‡ schedule for participants ≥12 months of age.

Table 8 Analysis Visit Windows for Coagulation, 7αC4, and sBA

Nominal Visit	Nominal Study Day	Lower Limit	Upper Limit
Baseline	1	(none)	1
Week 3	22	2	47
Week 10	71	48	84
Week 13	92	85	105
LTE Week 17	120	106	133
LTE Week K† (K is every 4 weeks after previous visit)	K*7+1	(K-2)*7+1	(K+2)*7
LTE Week L‡ (L is every 16 weeks after previous visit)	L*7+1	(L-8)*7+1	(L+8)*7

Note: † schedule for participants <12 months of age. ‡ schedule for participants ≥12 months of age.

3.8.3. Selection of Data in the Event of Multiple Records in an Analysis Visit Window

Depending on the statistical analysis method, single values may be required for each analysis window. For example, change from baseline by visit usually requires a single value, whereas a time-to-event analysis would not require a single value per analysis window.

If multiple valid, nonmissing, continuous measurements exist in an analysis window, records will be chosen based on the following rules if a single value is needed:

- In general, the baseline value will be the last available nonmissing value available prior to the first dosing date of study drug
  - For sBA, C4 and CSS baseline will be defined as the mean of the 2 last nonmissing assessments before the first dose of maralixibat, or the last nonmissing assessment if only a single assessment is available
- For postbaseline values:
  - The record closest to the nominal day for that visit will be selected.
  - If there are 2 records that are equidistant from the nominal day, the later record will be selected.
  - If there is more than 1 record on the selected day, the average will be taken, unless otherwise specified.

If multiple valid, nonmissing, categorical measurements exist in an analysis window, records will be chosen based on the following rules if a single value is needed:

- For baseline, the last available record on or prior to the date of the first dose of study drug will be selected. If there are multiple records with the same time or no time recorded on the same day, the value with the lowest severity will be selected (e.g., normal will be selected over abnormal for safety ECG findings).

- For postbaseline visits, if there are multiple records with the same time or no time recorded on the same day, the value with the highest severity within the window will be selected (e.g., abnormal will be selected over normal for safety ECG findings).

Participants are expected to schedule visits every 4 weeks in the LTE portion of the study while they are <12 months of age. The first time they attend a visit after they are ≥12 months of age, the next in-person visit should be scheduled 16 weeks later. For example, a participant who is 11.5 months old at LTE Week 21 will still be scheduled for LTE Week 25. The next visit should be LTE Week 41. Analysis visit windows will follow the per-protocol visit schedule for summaries. All LTE visits will be presented in listings.

4. STUDY PARTICIPANTS

4.1. Enrollment and Disposition

A summary of participant enrollment will be provided by cohort for each study site and overall. The summary will present the number and percentage of participants enrolled. For each column, the denominator for the percentage calculation will be the total number of participants analyzed for that column.

A summary of participant disposition will be provided by cohort. This summary will present the number of participants screened, the number of participants enrolled, and the number of participants in each of the categories listed below:

- Safety Analysis Set
- Ongoing in Core Study Period
- Completed Core Study Period
- Did not complete Core Study with reasons for premature discontinuation
- Entered LTE
- Ongoing in LTE
- Completed LTE
- Did not complete LTE with reasons for premature discontinuation

Participants who did not meet the eligibility criteria for study entry but enrolled in the study will be summarized. The summary will present the number and percentage of participants who did not meet at least 1 eligibility (inclusion or exclusion) criterion and the number of participants who did not meet specific criteria based on the Safety Analysis Set. A by-participant listing will be provided for those participants who did not meet at least 1 eligibility (inclusion or exclusion) criterion. The listing will present the eligibility criterion(a) that participants did not meet and related comments, if collected.

For the status of study completion and reasons for premature discontinuation, the number and percentage of participants in each category will be provided. The denominator for the percentage calculation will be the total number of participants in the Safety Analysis Set corresponding to that column. In addition, a flowchart will be provided to depict the disposition.

The following by-participant listings will be provided by participant ID number in ascending order to support the enrollment and disposition summary tables:

- Reasons for premature study discontinuation
- Lot number of study drug

4.2. Demographics

Participant demographic variables (i.e., age, sex, race, and ethnicity) will be summarized using descriptive statistics for age and using number and percentage of participants for sex, race, and ethnicity. The summary of demographic data will be provided for the Safety Analysis Set.

A by-participant demographic listing, including the informed consent date, will be provided by cohort and participant ID number in ascending order.

4.3. Other Baseline Characteristics

Other baseline characteristics include height (cm), weight (kg), body mass index (BMI, kg/m^2), head circumference (cm), mid-upper-arm circumference (cm) and the corresponding z-scores for age and sex for all the previous measures, baseline pruritus assessments (including CSS and ItchRO[Obs]), and baseline levels of biochemical markers of cholestatis and liver disease (including total serum bile acids [sBA], AST, ALT, ALP, bilirubin [total and direct], and 7α hydroxyl-4-cholesten-3-one [7αC4]). Genotype will also be included in the baseline characteristics (for PFIC participants only). The summary of baseline characteristics will be provided for the Safety Analysis Set.

A by-participant listing of other baseline characteristics will be provided by cohort and participant ID number in ascending order.

4.4. Medical History

Medical history will be collected at and during screening for disease-specific and general conditions (i.e., conditions not specific to the disease being studied) and coded using Medical Dictionary for Regulatory Activities (MedDRA) version 22.1.

General medical history data will not be summarized but will be listed. Medical history will be coded using MedDRA version 22.1.

A by-participant listing of disease-specific and general medical history will be provided by cohort and participant ID number in ascending order.

In deriving the time since disease diagnosis, all partial dates of diagnosis will be identified, and the partial dates will be imputed as follows:

- If day and month are missing but year is available, then the imputed day and month will be the later between 01 Jan and the date of birth.
- If day is missing but the month and year are available, then the imputed day will be the later between first day of the month and the date of birth.
- Date will not be imputed if the year is missing.

4.5. Prior and Concomitant Treatments

Medications collected at screening and during the study will be coded using the 2020 March version of the World Health Organization (WHO) Drug dictionary (WHODrug), Anatomical Therapeutic Chemical (ATC) Level 2 for ATC drug class and Level 5 (clinical substance) for preferred term.

Prior medications are defined as any medications taken by a participant prior to the first dose of study drug.

4.5.1. Prior Treatments of Interest

The number of prior medications/therapies administered for underlying liver disease (ALGS or PFIC), treatment of pruritus, and treatment of cholestasis will be summarized using the number and percentage of participants for each type of prior medication/therapy using descriptive statistics based on the Safety Analysis Set.

4.5.2. Other Prior Medications

Prior medications will be summarized ATC drug class and preferred term using the number and percentage of participants for each indication. A participant reporting the same medication more than once will be counted only once when calculating the number and percentage of participants who received that medication. The summary will be ordered alphabetically by ATC drug class and then by preferred term in order of descending overall frequency within each ATC drug class. For drugs with the same frequency, sorting will be performed alphabetically.

For the purposes of analysis, any medication with a start date prior to the first dose of study drug will be included in the prior medication summary regardless of the stop date. If a partial start date is entered, the medication will be considered prior unless the month and year (if day is missing) or year (if day and month are missing) of the start date are after the first dose of study drug. Medications with a completely missing start date will be included in the prior medication summary, unless otherwise specified.

Medications initiated during screening will be flagged and may also be summarized separately.

Summaries will be based on the Safety Analysis Set. No formal statistical testing is planned.

4.5.3. Concomitant Medications

Concomitant medications are defined as medications taken after the first dose of study drug. Use of concomitant medications will be summarized by ATC drug class and preferred term using the number and percentage of participants for each indication. A participant reporting the same medication more than once will be counted only once when calculating the number and percentage of participants who received that medication. The summary will be ordered alphabetically by ATC drug class and then by preferred term in descending overall frequency within each ATC drug class. For drugs with the same frequency, sorting will be performed alphabetically.

For the purposes of analysis, any medications with a start date prior to or on the first dose of study drug and continued to be taken after the first dose of study drug or started after the first dose of study drug but prior to or on the last dose date of study drug will be considered concomitant. Medications started and stopped on the same day as the first dose of study drug or the last dose of study drug will also be considered concomitant. Medications with a stop date prior to the date of first dose of study drug or a start date after the last dose of study drug will be excluded from the concomitant medication summary. If a partial stop date is entered, any medication with the month and year (if day is missing) or year (if day and month are missing) prior to the date of first dose of study drug will be excluded from the concomitant medication summary. If a partial start date is entered, any medication with the month and year (if day is missing) or year (if day and month are missing) after the study drug stop date will be excluded from the concomitant medication summary. Medications with completely missing start and stop dates will be included in the concomitant medication summary, unless otherwise specified. Summaries will be based on the Safety Analysis Set. No formal statistical testing is planned.

All prior and concomitant medications (other than per-protocol study drugs) will be provided in a by-participant listing sorted by cohort, participant ID number, and administration date in chronological order.

4.6. Extent of Study Drug Exposure

Treatment exposure will be summarized as starting dose, average dose, and ending dose across the treatment period. Descriptive statistics for these exposure measures will be provided by cohort and overall.

4.6.1. Duration of Exposure to Study Drug

Total duration of exposure to study drug will be defined as last dosing date minus first dosing date plus 1, regardless of any temporary interruptions in study drug administration except for protocol-specified dose holds, and will be expressed in weeks using up to 1 decimal place (e.g., 4.5 weeks). If the last study drug dosing date is missing, the latest date among the study drug end date, clinical visit date, laboratory sample collection date, and vital signs assessment date that occurred during the on-treatment period will be used.

The total duration of exposure to study drug will be summarized using descriptive statistics and using the number (i.e., cumulative counts) and percentage of participants exposed through the following time periods:

- 1 day, 3 weeks, 6 weeks, 10 weeks, 13 weeks, 17 weeks, 21 weeks, 24 weeks, 36 weeks, 48 weeks, and every 16 weeks thereafter.

A participant will be assumed to have completed x weeks of study drug if he or she has dosed for the lower bound of the protocol-specified visit window (3 days prior to the expected date). Summaries will be provided by cohort for the Safety Analysis Set.

No formal statistical testing is planned.

A listing of exposure to the study drug will be provided.

4.7. Protocol Deviations

Participants who did not meet the eligibility criteria for study entry but enrolled in the study will be summarized. The summary will present the number and percentage of participants who did not meet at least 1 eligibility (inclusion or exclusion) criterion and the number of participants who did not meet specific criteria based on the Safety Analysis Set. A by-participant listing will be provided for those participants who did not meet at least 1 eligibility (inclusion or exclusion) criterion. The listing will present the eligibility criterion(a) that participants did not meet and related comments, if collected.

Protocol deviations that occurred after participants entered the study are documented during routine monitoring. The number and percentage of participants with important protocol deviations by deviation reason (e.g., nonadherence to study drug, violation of select key inclusion/exclusion criteria) will be summarized by indication for the Safety Analysis Set. A by-participant listing will be provided for those participants with important protocol deviations.

5. STUDY ANALYSES

5.1. Adverse Events and Deaths

Summaries of adverse events will be presented overall and not by the portion of the study in which they occurred; that is, there will be no separate outputs for Core Study Period and LTE.

5.1.1. Adverse Event Dictionary

AEs will be coded using MedDRA v22.1 or later. System organ class (SOC), high-level group term (HLGT), high-level term (HLT), preferred term (PT), and lower-level term (LLT) will be provided in the AE dataset.

5.1.2. Adverse Event Severity

AE are graded by the investigator as Grade 1, 2, 3, 4, or 5 according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. The severity grade of events for which the investigator did not record severity will be categorized as "missing" for tabular summaries and data listings. The missing category will be listed last in summary presentation.

5.1.3. Relationship of Adverse Events to Study Drug

Related AEs are those for which the investigator selected "Related" on the AE eCRF to the question of "Relationship to Study Drug(s)." Relatedness will always default to the investigator's choice, not that of the medical monitor. Events for which the investigator did not record relationship to study drug will be considered related to study drug for summary purposes; however, by-participant data listings will show the relationship as missing.

5.1.4. Serious Adverse Events

SAEs will be identified and captured as SAEs if the AEs met the definitions of SAEs specified in the study protocol. SAEs captured and stored in the clinical database will be reconciled with the safety database before data finalization.

5.1.5. Treatment-Emergent Adverse Events

5.1.5.1. Definition of Treatment-Emergent Adverse Events

AE will be considered treatment emergent if the AE starts or worsens on or after the first dose of study drug and and no later than 14 days following the last dose of study drug.

For any participants who die during the study and the date of death is between the date of first dose of study drug and the date of study discontinuation (as entered by the site), inclusive, all AEs (including those resulting in death) that occur during the study will be considered as TEAEs irrespective of the last dose of study drug and will be included in the TEAE summaries.

5.1.5.2. Incomplete Dates

If the onset date of the AE is incomplete and the AE stop date is not prior to the first dosing date of study drug, then the month and year (or year alone if month is not recorded) of onset determine whether an AE is treatment emergent. The event is considered treatment emergent if both of the following 2 criteria are met:

- The AE onset is the same as or after the month and year (or year) of the first dosing date of study drug, and
- The AE onset date is the same as or before the month and year (or year) of the date corresponding to 14 days after the date of the last dose of study drug.

An AE with completely missing onset and stop dates or with the onset date missing and a stop date later than the first dosing date of study drug will be considered to be treatment emergent. In addition, an AE with the onset date missing and incomplete stop date with the same or later month and year (or year alone if month is not recorded) as the first dosing date of study drug will be considered treatment emergent.

5.1.6. Summaries of Adverse Events and Deaths

Treatment-emergent AEs will be summarized based on the Safety Analysis Set.

A brief, high-level summary of AEs will be provided by the number and percentage of participants who experienced them. The summary will include the total number and percent of participants reporting:

- Any TEAEs
- Any treatment-related TEAE
- Any severe TEAE
- Any severe treatment-related TEAE
- Any serious TEAE
- Any serious treatment-related TEAE
- Any TEAE that leads to permanent study drug discontinuation
- TEAEs that result in death

5.1.6.1. Summaries of AE Incidence

The number and percentage of participants who experienced AEs described below will be provided:

- TEAEs by SOC and PT
- Treatment-related AEs by PT
- Serious TEAEs by PT

Multiple events will be counted only once per participant in each summary. That is, if a participant experiences the same PT multiple times, only a single count will be added to the total. AEs will be summarized and listed first in alphabetical order of SOC and then by PT in descending order of total frequency within each SOC. A list of adverse events of special interest (AESIs) appears in Appendix 2.

In addition to the above summary tables, AESIs will be summarized by PT only in descending order of total frequency within cohort.

Furthermore, a data listing will be provided for the following:

- All AEs, indicating SOC, PT, grade and whether
  - it is treatment emergent
  - it is treatment related
  - it is of special interest
  - it is serious
  - it led to death (i.e., outcome of death)
  - led to discontinuation of study drug

5.1.7. Liver-Associated Events

A listing for liver-associated events will be provided by cohort, participant ID number in ascending order, and time point in chronological order.

No formal statistical testing is planned.

5.1.8. COVID-19 Impact

A listing for COVID-19 impact will be provided by cohort, participant ID number in ascending order, and time point in chronological order.

5.2. Laboratory Evaluations

Laboratory data collected during the study will be analyzed and summarized using both quantitative and qualitative methods. Summaries of laboratory data will be provided for the Safety Analysis Set and will include 1) data collected up to the last dose of study drug plus 7 days for participants who have permanently discontinued study drug or 2) all available data at the time of the database snapshot for participants who were ongoing at the time of an interim analysis. The analysis will be based on values reported in conventional units. When values are below the LOQ, they will be listed as such, and the closest imputed value will be used for the purpose of calculating summary statistics as specified in Section 3.7. Hemolyzed test results will not be included in the analysis, but they will be listed in by-participant laboratory listings.

Three separate listings for laboratory test results will be provided by participant ID number and time point in chronological order for hematology, serum chemistry, and urinalysis.

No formal statistical testing is planned.

5.2.1. Summaries of Numeric Laboratory Results

Descriptive statistics will be provided by cohort for each laboratory test specified in the study protocol as follows:

- Baseline values
- Values at each postbaseline time point
- Change and percentage change from baseline at each postbaseline time point

Baseline values are defined in Section 3.8.3. Change from baseline to a postbaseline visit will be defined as the visit value minus the baseline value. The mean, median, Q1, Q3, minimum, and maximum values will be displayed to the reported number of digits; SD values will be displayed to the reported number of digits plus 1.

5.3. Body Weight, Height, and Vital Signs

Descriptive statistics will be provided by cohort for body weight, height, BMI, their corresponding z-scores, and vital signs (e.g., systolic and diastolic blood pressure, temperature, respiratory rate, and heart rate) as follows:

- Baseline value
- Values at each postbaseline time point
- Change from baseline at each postbaseline time point

A baseline value will be defined as the last available value collected on or prior to the date/time of first dose of study drug. Change from baseline to a postbaseline visit will be defined as the

postbaseline value minus the baseline value. Body weight and vital signs measured at unscheduled visits will be included for the baseline value selection.

In the case of multiple values in an analysis window, data will be selected for analysis as described in Section 3.8.3. No formal statistical testing is planned.

Potentially clinically important (PCI) values for vital signs are shown in Table 9.

Table 9 Potentially Clinically Important Values for Vital Signs

Parameter	**Flag**	**Criteria** Observed Value
Systolic blood pressure (mmHg)	High	≥104
	Low	≤72
Diastolic blood pressure (mmHg)	High	≥56
	Low	≤37
Heart rate (bpm)	High	≥160
	Low	≤100
Body temperature (◦C)	High	≥38
	Low	≤36.6

The number and proportion of participants with PCI values will be presented by cohort.

In addition to summarizing observed and change from baseline values, measurements will also be summarized as a z-score. Height, weight, and BMI z-scores are based on a participant's sex and age at each scheduled visit. For participants <24 months of age, the World Health Organization (WHO) growth charts will be used to derive z-scores (WHO 2000). For participants ≥24 months of age, the Center for Disease Control (CDC) growth charts will be used to derive z-scores (CDC 2000).

A by-participant listing of vital signs will be provided by participant ID number and time point in chronological order. Body weight, height, and BMI will be included in the vital signs listing, if space permits. If not, they will be provided separately. Because height is planned to be collected only at screening, it will only be listed.

5.4. Mid-Upper-Arm and Head Circumference

Descriptive statistics will be provided by cohort for mid-upper-arm and head circumference, as well as their corresponding z-scores, as follows:

- Baseline value
- Values at each postbaseline time point
- Change from baseline at each postbaseline time point

A baseline value will be defined as the last available value collected on or prior to the date/time of first dose of study drug. Change from baseline to a postbaseline visit will be defined as the postbaseline value minus the baseline value. Values measured at unscheduled visits will be included for the baseline value selection.

5.5. Electrocardiogram Results

All ECGs will be 12-lead. ECG assessments will be listed in chronological order by participant ID for any participant with an abnormal (not clinically significant or clinically significant) tracing and overall.

Summaries of investigator assessment of ECG readings will be provided for the Safety Analysis Set for each scheduled time point. No formal statistical testing is planned.

5.6. Patient-Reported Outcomes

Exploratory endpoints (see Section 1.1.3) are designed to evaluate the impact of maralixibat on pruritus in study participants with pruritus at baseline over time.

- Change from baseline in morning ItchRO(Obs) severity score
- Change from baseline in evening ItchRO(Obs) severity score
- Change from baseline in highest daily ItchRO(Obs) severity score (defined as maximum of the morning and evening score on a given day)
- Change from baseline in Clinician Scratch Scale (CSS) score

The mean change in the average morning ItchRO(Obs) severity score between baseline and each visit will be calculated using weekly average morning ItchRO(Obs) severity scores.

Average morning ItchRO(Obs) severity scores will be calculated as the sum of the morning ItchRO(Obs) severity scores divided by the number of morning ItchRO(Obs) severity scores for the 7 days prior to each clinic visit. At least 4 of the 7 daily ItchRO(Obs) scores for a 7day period are required. If fewer than 4 daily ItchRO(Obs) scores are available, the average morning ItchRO(Obs) severity score at that visit will be treated as missing.

The same definitions will be used for the ItchRO(Obs) evening severity score and the daily ItchRO(Obs) score, which is defined as the maximum of the morning and evening score on a given day.

5.6.1. Summaries of Patient-Reported Outcomes

Descriptive statistics will be provided by cohort for each PRO specified in the study protocol as follows:

- Baseline values
- Values at each postbaseline time point
- Change from baseline at each postbaseline time point

Baseline values are defined in Section 3.8.3. Change from baseline to a postbaseline visit will be defined as the visit value minus the baseline value. The mean, median, Q1, Q3, minimum, and maximum values will be displayed to the reported number of digits; SD values will be displayed to the reported number of digits plus 1.

5.7. Pharmacokinetic Analyses

Due to poor absorption of maralixibat, very low systemic exposure and plasma drug levels are expected. Maralixibat plasma concentrations will be summarized using descriptive statistics by analysis visit.

Plasma maralixibat concentrations will also be presented in participant listings.

5.8. Other Measures

5.8.1. Health Utilization Assessments

Healthcare resource utilization variables include the number of hospitalizations, emergency ward visits, the length of stay for hospitalization (days), surgeries and procedures related to the participant's disease type, and the number of days the caregiver missed from work due to healthcare resource utilization events. Descriptive statistics including number of observations, mean, 95% CI on the mean, median, minimum, and maximum will be presented by visit for continuous variables. For categorical variables, the number and proportion of participants will be presented.

5.8.2. Liver Transplant List Status

A listing for liver transplant list status will be provided by cohort, participant ID number in ascending order, and time point in chronological order.

5.8.3. Physical and Neurodevelopmental Examination

All physical and neurodevelopmental examinations will be listed in chronological order by participant ID for any participant with an abnormal (not clinically significant or clinically significant) assessment and overall.

5.9. Changes From Protocol-Specified Analyses

There are no deviations from the protocol-specified analyses.

6. REFERENCES

CDC (2000). Centers for Disease Control growth charts – A SAS program for the 2000 CDC growth charts (ages 0 to <20 years) (www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm)

WHO (2000). World Health Organization growth charts – A SAS program for the WHO growth charts (ages 0 to <2 years) (www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas-who.htm)

7. SOFTWARE

SAS® Software Version 9.4. SAS Institute Inc., Cary, NC, USA.

8. SAP REVISION

Revision Date (DD MMM YYYY)	Section	Summary of Revision	Reason for Revision

9. APPENDIX 1 SCHEDULE OF ACTIVITIES

The schedule of activities changed from the original protocol to subsequent amendments. The original protocol and the latest amendment (Amendment 5) are shown.

Table 10 Schedule of Activities: Original Protocol

Procedure	Screening [a]	Dose Escalation (2-6 weeks in duration)							Stable Dosing (9-12 weeks in duration)						Core Study Final Visit	LTE (Week 14+) [b]		Follow-Up
Visit/PC Number	**Scr/V0**	**Bas/V1**	**V2**	**V3 [c]**	**V4**	**V5 [c]**	**V6**	**V7**	**PC**	**V8**	**PC**	**V9**	**PC**	**PC**	**V10[d]/ ET**	**PC**	**Vx**	**PC**
Study Week	**-4**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**+2**	**+2**	**+1**
Study Day	**Day -28 to -1**	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**91**	**+14**	**+14**	**+7 [e]**
Window (in days)	**(-5)**		**(±2)**	**(±2)**	**(±2)**	**(±2)**	**(±2)**	**(±2)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**
Informed consent	X																	
Eligibility assessment	X	X																
Demographics	X																	
Medical history	X																	
Physical examination & vital signs [f]	X	X	X	X	X	X	X	X		X		X			X		X	
Midarm and head circ [g]		X								X					X			
Neurodevelopmental assessment		X													X			
12-lead ECG [h]		X													X			
Clinician Scratch Scale		X								X					X		X	
CBC with differential [i]	X						X								X		X	
Coagulation [i]	X	X			X							X			X		X	
Chemistry panel [i]	X	X			X		X			X		X			X		X	
7αC4 [i]	X						X								X			
sBA collection [i, j]		X													X		X	
Lipid soluble vitamins [i, j]	X							X							X		X	
PK sample [k]		X								X					X			
Healthcare utilization		X													X			X
Dispense maralixibat [l, m]		X	X		X		X	X		X		X			X		X	

Maralixibat administration [m]		X	
Assess AEs [n]		X	
Prior and concomitant treatment(s)		X	

7αC4=7α-hydroxy-4-cholesten-3-one; AE=adverse event; ALGS=Alagille syndrome; Bas=baseline; CBC=complete blood count; circ=circumference; ET=early termination; LTE=long-term extension; PC=participant contact; PEBD=partial external biliary diversion; PFIC=progressive familial intrahepatic cholestasis; PK=pharmacokinetic; RBP=retinol binding protein; sBA=serum bile acid; Scr=screening; V=visit.

[a] Participants who initially do not meet eligibility criteria may be reassessed during the 4-week screening period prior to being recorded as a screen failure. Participants may also be rescreened.

[b] During the LTE, contact with participants will occur every other week, with alternating clinic visits and phone calls.

[c] May be phone calls, at the investigator's discretion. Physician examination and vital signs required only if a clinical visit takes place. AEs and concomitant medications to be recorded via phone call.

[d] Study sites should record dates of any future scheduled procedures related to PFIC or ALGS (e.g., PEBD, ileal exclusion, liver transplant, or listed for liver transplant), if known at this visit.

[e] Follow-Up contact is not needed if ET visit took place more than 7 days after the last dose.

[f] Blood pressure, heart rate, temperature, and respiration rate. Length and weight will be measured by trained staff using standardized methodology, including calibrated headboard (infantometer) and calibrated infant scale, respectively.

[g] Midarm circumference should be measured on the same arm throughout the study.

[h] ECGs will be taken in 12-lead, triplicate at baseline and Visit 10, with PK samples taken during the same visit.

[i] Results may be taken from existing laboratory assessments if blood is drawn within 5 days of the study clinic visit.

[j] Samples should be taken before feeding, approximately 2 hours from a feeding before or after blood collection, when possible. Fluid intake, excluding milk, is permitted if necessary. For participants <7 kg, lipid-soluble vitamin values can be taken from assessments made as part of standard of care (±14 days). If blood volumes do not allow all assessments within a single visit, a separate clinical visit in which samples for retinol, α-tocopherol, vitamin K, and RBP as well as 25-hydroxy vitamin D are taken can be made.

[k] Blood samples should be drawn before feedings and administration of vitamin supplementation, when possible. Systemic concentrations of maralixibat in plasma will be determined at predose and at approximately 2.5 hours (±30 minutes) after dosing.

[l] If needed, maralixibat may be supplied via direct-to-participant shipments between site visits.

[m] Participants will be administered the first dose of maralixibat in the clinic at Visit 1/Study Day 0 under the supervision of the investigator or trained site staff. Subsequently, the morning dose should be administered approximately 30 minutes before the first meal of the day where possible and, for children with PFIC, approximately 30 minutes before the main evening meal/feeding. Caregivers will be instructed to complete the dosing diary accordingly.

[n] Adverse events and adverse events of special interest will be collected from Study Day 0. Serious adverse events will be recorded from the signing of the Informed Consent Form.

Table 11 Schedule of Activities: Amendment 5

Procedure	Screening [a]	Dose Escalation (2-6 weeks in duration)							Stable Dosing (9-12 weeks in duration)						Core Study Final Visit	LTE (Week 15+, <12 months of age; 4-week repeating cycle)		LTE (≥12 months of age; 16-week repeating cycle)	LTE Final Visit[b]	Follow-Up
Visit/PC Number	**Scr/V0**	**Bas/V1**	**V2[c]**	**V3[c]**	**V4**	**V5[c]**	**V6[c]**	**V7**	**PC**	**PC**	**PC**	**V8**	**PC**	**PC**	**V9[d]/ ET**	**PC**	**Vx**	**Vx**	**Vx/ET**	**PC**
Study Week	**-4**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**+2**	**+2**	**+16**		**+1**
Study Day	**Day -28 to -1**	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**91**	**+14**	**+14**	**+112**		**+7[e]**
Window (in days)	**(-5)**		**(±2)**	**(±2)**	**(±2)**	**(±2)**	**(±2)**	**(±2)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±3)**	**(±14)**	**(±14)**	**(±3)**
Informed consent	X														X[f]				X[f]	
Eligibility assessment	X	X																		
Demographics	X																			
Medical history[g]	X																			
Physical examination & vital signs [h]	X	X	X	X	X	X	X	X				X			X		X	X	X	
Mid-upper-arm and head circumferences [i]		X						X				X			X		X	X	X	
Neurodevelopmental assessment [j]		X													X			X[j]		
12-lead ECG [k]		X													X					
ItchRO(Obs)[l]	X[l]	X[l]			X[l]			X[l]				X[l]			X[l]		X[l]		X[l,m]	
Clinician Scratch Scale	X	X						X							X		X	X	X	
CBC with differential [n]	X							X							X		X	X	X	
Coagulation [n]	X	X			X							X			X		X	X	X	
Chemistry panel [n]	X	X			X			X				X			X		X	X	X	
7αC4 [n]		X			X							X			X		X	X	X	
sBA collection[n,o]	X	X			X							X			X		X	X	X	
Lipid-soluble vitamins[n,o]	X							X							X		X	X	X	
Lipid panel [n, o]	X	X			X			X				X			X		X	X	X	
PK sample [p]		X						X				X			X					
Healthcare resource utilization		X													X		X	X	X	X
Dispense maralixibat [q,r]		X	X		X		X	X				X			X		X	X		
Maralixibat administration [r]		X																		

Assess AEs [s]	X
Prior and concomitant treatment(s)	X

7αC4=7α-hydroxy-4-cholesten-3-one; AE=adverse event; AESI=adverse event of special interest; ALGS=Alagille syndrome; Bas=baseline; CBC=complete blood count; circ=circumference; eCRF=electronic case report form; ET=early termination; ItchRO(Obs)=Itch Reported Outcome Observer; LTE=long-term extension; MUAC=mid-upper-arm circumference; PC=participant contact; PEBD=partial external biliary diversion; PFIC=progressive familial intrahepatic cholestasis; PK=pharmacokinetic; RBP=retinol binding protein; SAE=serious adverse event; sBA=serum bile acid; Scr=screening; V=visit.

[a] Participants who initially do not meet eligibility criteria may be reassessed during the 4-week screening period prior to being recorded as a screen failure. Participants may also be rescreened with medical monitor approval.

[b] Only consent (and assent as appropriate) to check disease progression and ItchRO(Obs) (if <12 months of age) will be completed if the participant has attended a clinic visit ≤8 weeks prior to the final LTE visit/ET.

[c] May be phone calls, at the investigator's discretion. Physician examination and vital signs required only if a clinical visit is conducted. AEs and concomitant medications to be recorded from information received via phone call.

[d] Study sites should record dates of any future scheduled procedures related to PFIC or ALGS (e.g., PEBD, ileal exclusion, liver transplant, or listed for liver transplant), if known at this visit.

[e] Follow-up contact is not needed if ET visit was conducted >7 days after the last dose.

[f] Participants who provide consent (and assent as appropriate) and discontinue the study during the core or LTE will be followed up at least once yearly (from the last study visit) to check disease progression.

[g] Medical history is to include conditions the participant had prior to the first dose of study drug, with the exception of pretreatment SAEs, which should be reported on the AE eCRF.

[h] Blood pressure, heart rate, temperature, and respiration rate. Length and weight will be measured by trained staff using standardized methodology, including calibrated headboard (infantometer) via 2 independent measurements and recorded to the nearest 0.1 cm, and calibrated infant scale via 2 independent measurements and recorded to the nearest 0.1 kg, respectively.

[i] Mid-upper-arm circumference should be measured with a MUAC tape via 3 independent measurements recorded to the nearest 0.1 cm on the same arm throughout the study. Head circumference will be measured 3 times, and the largest measurement recorded to the nearest 0.1 cm.

[j] A full neurological examination, including neurodevelopmental milestones, will be performed using standardized instructions provided to the study site. Once the participant is ≥12 months of age, the assessment only needs to be performed once per year.

[k] 12-lead ECGs will be taken in triplicate.

[l] Caregivers complete ItchRO(Obs) severity questionnaires twice daily for at least 7 consecutive days in the 2 weeks prior to the baseline visit and for 7 days prior to other study clinic visits.

[m] ItchRO(Obs) is only assessed at the LTE final visit if participant is <12 months of age.

[n] Results may be taken from existing laboratory assessments if blood is drawn within the specified number of days of the study clinic visit, indicated within the Laboratory Manual (see Laboratory Manual for details). For clinical chemistry, screening results may not replace baselineassessments.

[o] Samples should be taken before feeding, ~2 hours after food or formula, and before administration of vitamin supplementation, when possible. Water intake, excluding milk, is allowed when required. For participants <7 kg, lipid-soluble vitamin values can be taken from assessments made as part of standard of care (±14 days). If blood volumes do not allow all assessments within a single visit, a separate clinical visit in which samples for retinol, α-tocopherol,and 25-hydroxy vitamin D are taken can be made.

[p] Systemic concentrations of maralixibat in plasma will be determined at predose and at ~2.5 hours (±30 minutes) after dosing. Predose samples are not required for measures made at baseline.
[q] If needed, maralixibat may be supplied via direct-to-participant shipments between site visits.
[r] Participants will be administered the first dose of maralixibat in the clinic at Visit 1/Study Day 0 under the supervision of the investigator or trained site staff. Subsequently, the morning dose should be administered ~30 minutes before the first meal of the day where possible and, for children with PFIC, ~30 minutes before the main evening meal/feeding. Caregivers will be instructed to complete the dosing diary accordingly.
[s] AEs and AESIs will be collected from the first dose of study drug. SAEs will be recorded from the signing of the Informed Consent Form.

10. APPENDIX 2 ADVERSE EVENTS OF SPECIAL INTEREST

The following events are AESIs for participants in this study:

- Lipid-soluble vitamin deficiency requiring study drug discontinuation
- Liver parameter disruption requiring study drug interruption and/or dose modification
- Any events that are suspected and/or confirmed to be due to propylene glycol toxicity, including but not limited to neurological complications, hemolysis, and cardiac arrhythmias

The following Medical Dictionary for Regulatory Activities (MedDRA) preferred terms are used to define the above concepts:

Lipid-Soluble Vitamin Deficiency

Vitamin A decreased	Vitamin E deficiency
Vitamin A abnormal	Vitamin K decreased
Vitamin A deficiency	Vitamin K deficiency
Vitamin D decreased	International normalised ratio increased
Vitamin D abnormal	International normalised ratio abnormal
Vitamin D deficiency	Blood 1,25-dihydroxy vitamin D decreased
Vitamin E decreased	

Liver Parameter Disruption

Alanine aminotransferase abnormal	Gamma-glutamyltransferase abnormal
Alanine aminotransferase increased	Gamma-glutamyltransferase increased
Aspartate aminotransferase abnormal	Hepatic enzyme abnormal
Aspartate aminotransferase increased	Hepatic enzyme increased
Bilirubin conjugated abnormal	Liver function test abnormal
Bilirubin conjugated increased	Liver function test increased
Blood bilirubin abnormal	Transaminases abnormal
Blood bilirubin increased	Transaminases increased
Blood bilirubin unconjugated increased	Hyperbilirubinemia

Propylene Glycol Toxicity

Propylene glycol toxicitiy will be identified via the MedDRA lower-level term (LLT) of Poisoning by pharmaceutical excipients.