PROTOCOL NUMBER: VLX-401

Title:	A Phase 2a/2b Randomized Double-Blind Placebo-Controlled Study to Evaluate the Efficacy and Safety of V**o**lixibat in Adult Women with Intra**h**epatic Cholestasis of Pregnancy and Elevated Serum Bile **A**cid Co**n**centr**a**tions (OHANA)
Study Treatment:	Volixibat (formerly SHP626, LUM002)
Regulatory Agency Identifying Numbers:	
IND:	147904
EudraCT:	2020-003448-96
Phase:	2a/2b
Sponsor:	Mirum Pharmaceuticals, Inc. 950 Tower Lane Foster City, CA 94404
Original Protocol	Version 1, 14 October 2020
Protocol History:	Version 1 (United Kingdom), 4 December 2020 Version 2, 18 May 2021 Version 3, 7 October 2021 Version 4, 20 April 2022

This study will be performed in compliance with the International Council for Harmonisation Good Clinical Practices and applicable regulatory requirements, including the archiving of essential documents.

INVESTIGATOR'S ACKNOWLEDGEMENT

I have read this protocol for Mirum Pharmaceuticals, Inc., Study VLX-401, Version 4.

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I have fully discussed the objectives of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a participant to obtain his or her consent (and/or assent when appropriate) to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines on Good Clinical Practice (GCP) and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study, I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address:	

Signature: _____ **Date:** _____

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1.1. Synopsis

Name of Sponsor/Company: Mirum Pharmaceuticals, Inc.	
Name of Investigational Product: Volixibat	
Name of Active Ingredient: Volixibat potassium (formerly SHP626 and LUM002)	
Protocol Number: VLX-401	
Title of Study: A Phase 2a/2b Randomized Double-Blind Placebo-Controlled Study to Evaluate the Efficacy and Safety of V**o**lixibat in Adult Women with Intra**h**epatic Cholestasis of Pregnancy and Elevated Serum Bile **A**cid Co**n**centr**a**tions (OHANA)	
Study Centers: This study will be conducted at multiple sites across Australia, New Zealand, North America, and the United Kingdom. Other regions may be added.	
Participants: Up to 280 participants will be enrolled in this study. • Part 1 (open label): Up to 80 participants with serum bile acid (sBA) ≥10 μmol/L during the screening period or at any time during the current pregnancy will be enrolled. • Part 2 (double blind): Approximately 200 participants with a screening sBA level ≥20 μmol/L will be enrolled (100 per arm).	
Randomization: Part 1 (open label): • 1:1 (volixibat 20 mg twice daily [BID]/volixibat 80 mg BID) Part 2 (double blind): • 1:1 (selected volixibat dose BID/placebo BID) Randomization will be stratified according to background use of ursodeoxycholic acid (UDCA) or not (in Part 1), gestational age at randomization, presence/absence of gestational diabetes, and highest sBA levels before randomization (in Part 2).	
Study Period: 2020 to 2024	**Study Phase:** 2a/2b

Objectives and Endpoints

Primary

Part 1:

- To assess the safety and tolerability of volixibat in participants with ICP on the basis of the following endpoints:
  - Proportion of participants experiencing one or more of the following:
    - Treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), adverse events of special interest (AESIs), events of clinical interest (ECIs), and adverse events (AEs) that lead to discontinuation of study drug
    - Clinically significant laboratory abnormalities

Part 2:

- To assess the efficacy of volixibat on the reduction of elevated sBA concentrations in participants with intrahepatic cholestasis of pregnancy (ICP) on the basis of the following endpoint:
  - Mean change from baseline to Week 3 of the treatment period in total sBA concentration

Secondary

The following secondary objectives and endpoints are applicable in Part 1 and Part 2.

- To assess the efficacy of volixibat on pruritus due to ICP on the basis of the following endpoint:
  - Mean change from baseline to Week 3 of the treatment period in the weekly average worst daily itch score as measured by the Adult Itch Reported Outcome (ItchRO)
- To assess the impact of volixibat on a composite perinatal outcome in participants with ICP on the basis of the following endpoints:
  - Proportion of participants experiencing one or more of the following:
    - Perinatal death, defined as in utero fetal death after randomization or known neonatal death up to 28 days after birth
    - Spontaneous preterm delivery, defined as delivery at <37 weeks' gestational age following spontaneous onset of preterm labor or preterm premature rupture of the membranes (PPROM)
    - Iatrogenic preterm delivery attributable to ICP or ICP-related complications, defined as delivery at <37 weeks' gestational age resulting from medical intervention (i.e., augmentation/induction of labor or cesarean delivery without prior spontaneous onset of preterm labor or PPROM with one or more of the following as the indication for delivery):
      - Diagnosis of ICP with or without suspected fetal compromise
      - Persistently elevated/increasing sBA
      - Worsening hepatic function
      - Intolerable maternal ICP symptoms
    - Neonatal unit admission for ≥12 hours from birth until hospital discharge for one or more of the following indications:
      - Respiratory insufficiency/failure with requirement for oxygen supplementation
      - Noninvasive or invasive respiratory support

- Hemodynamic instability or shock requiring clinical intervention
- Metabolic acidosis or signs of asphyxia
- Proven infection
- Hypoglycemia or feeding problems requiring intravenous (IV) fluids or tube feeding
- Meconium-stained amniotic fluid and/or its sequelae (e.g., meconium aspiration, etc.)

Safety

- To assess the safety and tolerability of volixibat in participants with ICP on the basis of the following endpoints in Part 2:
  - Incidence of TEAEs, SAEs, AESIs, ECIs, and AEs that lead to discontinuation of study drug
  - Incidence of clinically relevant laboratory abnormalities

Exploratory

The following exploratory objectives and endpoints are applicable to Part 1 and Part 2:

- To assess volixibat drug levels in maternal and fetal plasma and pharmacodynamic (PD) markers in maternal blood in participants with ICP on the basis of the following endpoints:
  - Volixibat concentrations in maternal and fetal plasma (from umbilical cord sampling)

    Note: Assessment of maternal/fetal volixibat drug levels is a secondary objective for Part 1.
  - Change in biomarkers of bile acid synthesis, inflammation, and pruritogens
  - Proportion of participants with sBA <40 μmol/L at end of the treatment period
- To assess the longer-term efficacy of volixibat in participants with ICP on the basis of the following endpoints:
  - Mean change from Week 3 to end of treatment period in total sBA concentration
  - Mean change from baseline to end of treatment period in total sBA concentration
  - Association between changes from baseline to Week 3 in total sBA and ItchRO as well as their association with the composite perinatal outcome
- To assess the effect of volixibat on additional perinatal outcomes on the basis of the following endpoints:
  - Mean gestational age at delivery
  - Proportion of participants with an early term delivery (37–38 weeks 6 days, inclusive)
  - Proportion of participants with a full-term delivery (≥39 weeks)
  - Proportion of participants experiencing a perinatal death
  - Proportion of participants with a spontaneous preterm delivery
  - Proportion of participants with an iatrogenic preterm delivery attributable to ICP
  - Proportion of neonates requiring neonatal intensive care unit (NICU) admission for ≥12 hours between birth and hospital discharge
  - Proportion of neonates with meconium-stained amniotic fluid at delivery
  - Proportion of participants requiring rescue medication for ICP

- Proportion of neonates with Apgar score <7 at 5 minutes of life
- Mean birth weight percentile
- Mean placental weight percentile
- Proportion of neonates with umbilical cord pH <7.0 at birth
- Means for umbilical cord blood sBA, total cholesterol, low-density lipoprotein cholesterol, and glucose
- Mean maternal estimated blood loss at delivery
- Mean time from randomization to delivery

- To evaluate the effect of volixibat on additional measures of pruritus efficacy and quality of life in participants with ICP on the basis of the following endpoints:
  - Change from baseline in the 5-D Itch Scale
  - Change from baseline in Clinician Scratch Scale (CSS)
  - Change from baseline in EuroQoL 5-dimension, 5-level (EQ-5D-5L)
  - Change from baseline in Patient-Reported Outcomes Measurement Information System (PROMIS®) Short Form (SF) Fatigue 7a
  - Change from baseline in PROMIS SF Sleep Disturbance
  - Change from baseline in Patient Impression of Severity-Itch
  - Patient Global Impression of Change-Itch
- To assess the impact of volixibat on healthcare utilization in participants with ICP on the basis of the following endpoints:
  - Number and duration of medical care encounters, including surgeries and other selected procedures (inpatient and outpatient)
  - Dates and duration of hospitalization (total days or length of stay, including duration by wards [e.g., intensive care unit])
  - Number and type of diagnostic and therapeutic tests and procedures
  - Outpatient medical encounters and treatments (including physician or emergency department visits, tests and procedures, and medications)

Study Design

This is an operationally seamless adaptive clinical study that will consist of 2 parts. Part 1 of the study is open label. Part 2 of the study constitutes a randomized, double-blind, placebo-controlled phase comparing the selected volixibat dose with placebo for superior efficacy.

Part 1: Proof of Concept, Safety/Tolerability, Dose Ranging, Pharmacokinetics, and Interim Analysis

After a screening period of up to 10 days, eligible participants diagnosed with ICP with screening sBA ≥10 µmol/L will be randomized with stratification (based on background use of UDCA or not, on gestational age [<32 weeks or ≥32 weeks] at randomization, and presence/absence of gestational diabetes) in a 2-arm (1:1), open-label fashion to receive volixibat 20 mg BID or volixibat 80 mg BID.

Study-drug dosing will begin on Day 0 and continue until end of the treatment period, defined as the day of delivery. Study visits to assess safety, plasma concentrations of volixibat, pharmacodynamics, and efficacy will be conducted from baseline (Day 0) until delivery at time points specified in the protocol. Because alternative measures may be implemented when necessary for public health or other emergency situations, and when sufficient to help ensure the safety of study participants, some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. Participants will also enter ItchRO assessments into an electronic diary (eDiary) once daily (QD). Use of limited rescue therapies for ICP will be permitted for participants who meet protocol-specified criteria.

Part 2: Confirmation of Safety and Efficacy with Selected Dose

For Part 2, it is anticipated that ~200 participants (100 per arm) will be randomized after the volixibat dose is selected in Part 1.

Participants with ICP with a screening sBA level ≥20 μmol/L who also meet the ItchRO criteria will be randomized with stratification (based on gestational age [<32 weeks or ≥32 weeks] at randomization, presence/absence of gestational diabetes, and highest sBA before randomization [<40 μmol/L, ≥40 μmol/L to <100 μmol/L, or ≥100 μmol/L]) in a 2-arm (1:1), double-blind fashion to receive the selected volixibat dose BID or placebo.

Study visits to assess safety, pharmacodynamics, efficacy, and plasma concentrations of volixibat in all participants will be conducted from baseline (Day 0) until delivery at time points specified in the protocol; sampling for volixibat concentrations may be discontinued in Part 2 if Part 1 results demonstrate consistently negligible or below the limit of quantification concentrations in maternal and umbilical cord (fetal) plasma. Because alternative measures may be implemented, when necessary, for public health or other emergency situations and when sufficient to help ensure the safety of study participants, some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. Participants will also enter ItchRO assessments into an eDiary QD. Use of limited rescue therapies for ICP will be permitted for participants who meet protocol-specified criteria.

Safety

In both Part 1 and Part 2, safety assessments will include AEs, clinical laboratory tests, vital signs, and physical examinations. Additional fetal safety assessments will include serial ultrasounds to assess fetal growth and nonstress tests (NSTs)/cardiotocography (CTG) or fetal biophysical profile (BPP). Assessment of plasma concentrations of volixibat will be obtained in maternal plasma and umbilical cord plasma (when possible) at single time points, with no formal pharmacokinetic (PK) parameter analysis planned. PD assessments will include bile acids alongside other biomarkers.

In Part 1, ongoing monitoring of open-label safety data will be conducted by the sponsor at regular intervals per the medical and data monitoring plans as well as the routine safety surveillance activities, including but not limited to signal detection monitoring. Additionally, a chartered, independent data monitoring committee (IDMC) will review unblinded maternal and fetal safety/plasma drug level data on an ongoing basis during this study (Part 2 only), with the initial IDMC meeting (post-study start) to be held after 12 participants in Part 2 complete the study; cadence for subsequent meetings will be outlined in the IDMC charter. The sponsor plans to continue formal safety and efficacy monitoring via a chartered IDMC in Part 2; this will include AE monitoring specifications.

End of Study

The end of the study is defined as the date of the last visit of the last participant in the study. The end of study for individual participants is the completion of the follow-up period after the day of delivery, including completion of follow-up Visit 2 (F2).

Inclusion Criteria

Participants who meet all of the following criteria will be eligible for the study:

Informed Consent

1. Provide signed informed consent as described in the protocol and be willing to comply with all study visits and requirements through end of study, including the follow-up period (F2)

Age

2. Female aged ≥18 and ≤45 years with viable pregnancy of 20 weeks 0 days or above:

   Part 1: Singleton gestation and no more than 37 weeks 0 days (inclusive) or twin gestation and no more than 35 weeks 0 days (inclusive) at the baseline visit (Day 0)

   Part 2: Singleton gestation and no more than 36 weeks 0 days (inclusive) at the baseline visit (Day 0)

   Note: Confirmation of eligibility based upon estimated gestational age required per National Institute for Health and Care Excellence (NICE) guidelines before randomization: crown-rump length (CRL) from early ultrasound or head circumference on ultrasound if CRL >84 mm.

Type of Participant and Disease Characteristics

3. Diagnosis of ICP, as characterized by the following:

   Onset of pruritus during pregnancy with no known etiology other than ICP (e.g., pruritic urticarial papules and plaques of pregnancy, pruritic folliculitis, uncontrolled thyroid disease, etc.)

   sBA level ≥10 µmol/L at any point during the current pregnancy

   Absence of known pathology that may produce similar laboratory findings/symptoms; these include but are not limited to other cholestatic disorders, such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC)

   Note: Suspicion of transient hypercholanemia should be discussed with medical monitor to confirm participants eligibility.

4. Part 1:

   sBA of ≥10 µmol/L as assessed by the central laboratory during the screening period or local laboratory at any time during the current pregnancy

   Part 2:

   sBA level ≥20 µmol/L as assessed by the central laboratory during the screening period

   Documentation of historic or local sBA level is required to confirm participants eligibility before randomization.

   Laboratory assessments may be repeated at investigator's discretion before randomization.

   Participants already on UDCA at the time of the screening visit shall be eligible for Part 1.

5. Willing and able to refrain from the use of any of the following for the duration of the study: statins, rifampin, bile acid sequestrants (e.g., cholestyramine), peroxisome proliferator-activated receptor (PPAR) agonists/fibrate drugs (e.g., fenofibrate, bezafibrate), S-adenosyl methionine

Participants requiring local/systemic corticosteroids or selective serotonin reuptake inhibitors (e.g., sertraline) for the management of non-cholestatic, non-pruritic conditions must have been on a stable dose for at least 1 week before screening and be willing to remain on a stable dose for the duration of the study to be considered eligible.

Administration of isolated doses of corticosteroids for obstetric indications (e.g., promotion of fetal lung maturity for anticipated preterm delivery) is permitted.

6. Part 2 only: Willing and able to enter daily patient-reported outcome information in an eDiary for the duration of the study and completes ≥70% of the eDiary ItchRO assessments during the screening period. A minimum of 4 once-daily ItchRO entries are required during screening.
7. Part 2 only: For the primary cohort only: Qualified pruritus reflected by an average daily Adult ItchRO score ≥4 overall during the screening period

Exclusion Criteria

Any one of the following will exclude potential participants from eligibility for the study:

Medical Conditions

1. At the time of either the screening or baseline visit, decision has already been made to deliver within the next 7 days, for any indication
2. Presence of triplets or higher multiple gestation, known placenta accreta, complete placenta previa, premature rupture of membranes (PROM) at any gestational age prior to randomization, cervical incompetence, history of prior spontaneous birth at ≤34 weeks not secondary to known or suspected ICP, or other condition (in the opinion of the investigator/medical monitor [refers to sponsor or contract research organization medical monitor throughout]) likely to result in spontaneous or iatrogenic delivery before 37 weeks
3. Known non-reassuring fetal status based upon antepartum testing (e.g., NST/CTG or BPP) at or within 7 days before the baseline visit
4. Known fetal anomaly likely to result in intrauterine fetal demise or neonatal death within the first 30 days of life
5. History or evidence, with the exception of ICP, of current underlying cholestatic liver disease at screening or baseline, including but not limited to:
   - PSC or secondary sclerosing cholangitis (SSC)
   - PBC
   - Immunoglobulin G4-related cholangitis
   - Ascending cholangitis
   - Clinical evidence or suspicion of dominant strictures that are considered clinically relevant in the opinion of the investigator/medical monitor, or current or planned placement of percutaneous drain/biliary stent at screening
6. Evidence of other hepatobiliary conditions at screening or baseline, including but not limited to:
   - Active hepatitis A infection

- Active hepatitis B infection as defined by the presence of hepatitis B surface antigen or presence of hepatitis B virus DNA
- Hepatitis C as defined by the presence of hepatitis C virus (HCV) antibody and positive HCV RNA. Documented cured HCV infection is acceptable if >2 years earlier than the time of randomization
- History or evidence of autoimmune hepatitis, Wilson disease, alpha-1-antitrypsin deficiency, hemochromatosis, or drug-induced liver disease (as defined on the basis of typical exposure and history). A history of temporary, reversible cholestasis associated with medication use (e.g., hormones, antibiotics, etc.) is not exclusionary.
- Evidence or clinical suspicion of cirrhosis, including decompensated cirrhosis (e.g., hepatic encephalopathy, portal hypertension, hepato-renal syndrome, hepato-pulmonary syndrome, esophageal varices, ascites)
- Suspected or proven cholangiocarcinoma or hepatocellular carcinoma
- History of liver transplantation
- Confirmed or suspected nonalcoholic steatohepatitis as determined by the investigator
- Acute fatty liver of pregnancy
- Severe preeclampsia/hemolysis, elevated liver enzymes, and a low platelet count (HELLP) syndrome; suspected hemolytic anemia

  Note: The presence of mild preeclampsia, gestational hypertension, or chronic hypertension is not considered exclusionary for study participation if the condition is adequately managed and there is no known plan for delivery within the next 7 days.
- Alcohol-related liver disease
- Known diagnosis of Gilbert syndrome

  Note: The presence of gallstones or a prior history of cholecystectomy is NOT considered exclusionary.

7. History or presence of known or suspected inflammatory bowel disease (e.g., Crohn's, ulcerative colitis)
8. History of small bowel resection or bariatric surgery (e.g., gastric bypass, banding, or sleeve), or surgery resulting in disruption of the enterohepatic circulation
9. Part 2 only: Presence of any concurrent underlying condition with unstable pruritus (e.g., unstable atopic dermatitis, psoriasis, urticaria, psychogenic pruritus, etc.)

   Note: Participants with stable pruritic conditions (as above) that predate pregnancy and who have been on stable doses of treatment medications for the pruritic condition for ≥4 weeks before screening, may be considered eligible
10. Anticipated need for liver transplantation within 12 months (365 days) after randomization

11. Active infection that, in the opinion of the investigator or medical monitor, would preclude participation in the study for participant safety reasons

    Note: An active COVID-19 infection (or recent infection within past 15 days) is considered exclusionary unless approved by the medical monitor.

12. Unstable and/or serious medical disease that is likely to impair the participant's ability to participate in all aspects of the study or result in substantially shortened life expectancy (e.g., malignancy, end-stage heart failure)

13. Known diagnosis of HIV infection or HIV antibody positivity

14. Clinically relevant alcohol use disorder or substance abuse within 12 months (365 days) before screening. Alcohol use disorder for this study is defined as ≥2 standard drinks on average per day for women, with a standard drink defined as 1.5 oz (1 shot) of liquor, 5 oz of nonfortified wine, or 12 oz of beer (as defined by the National Institute of Alcohol Abuse and Alcoholism; 1 oz=29.57 mL).

Diagnostic Assessments

15. Participant has the following laboratory parameters at the screening visit, as determined by the central laboratory:

    - Platelet count ≤100,000/mm3
    - Serum creatinine ≥77 µmol/L (0.87 mg/dL)
    - AST or ALT ≥5 × ULN
    - Total bilirubin >1.3 mg/dL
    - INR >1.3 unless due to therapeutic anticoagulation

16. Participant has evidence or preexisting history of atrial fibrillation, atrial flutter, complete bundle branch or heart block, Wolff-Parkinson-White syndrome, or other abnormality that is deemed clinically significant and/or likely to put participants at unacceptable risk to study participation, as determined by the investigator or the medical monitor.

17. Except as defined elsewhere in the inclusion or exclusion criteria, abnormal laboratory results that are deemed to be clinically significant and put participants at unacceptable risk for study participation, as determined by the investigator or the medical monitor

NOTE: Investigators have the discretion to repeat assessments if they believe there is a reasonable possibility that the results were spurious or otherwise confounded. Repeat assessments (no more than 1 per laboratory parameter, under appropriate conditions) must be conducted within the screening period.

Prior/Concomitant Therapy

18. Use within 5 half-lives before randomization of any of the following medications:

    - Methotrexate
    - Sodium phenylbutyrate
    - PPAR agonists (e.g., fibrates)
    - Any investigational drug

- Known hepatotoxins (e.g., cyclosporine, tacrolimus, Janus kinase [JAK] inhibitors [when dosed systemically], pancreatic lipase inhibitors [when dosed systemically], drugs [including any medicinal products, herbs, etc.] that are known to cause drug-induced liver injury [DILI] at the therapeutic labeled doses)

19. Use within 5 half-lives before randomization of any of the following medications:
    - UDCA (Part 2 only; UDCA use is permitted in Part 1)
    - Rifampicin
    - Bile acid/lipid-binding resins (e.g., cholestyramine)
    - S-adenosylmethionine
    - Any other medications for the treatment of cholestasis – on- or off-label use
20. Use within 5 half-lives before randomization of any of the following (when dosed systemically):
    - Progesterone supplementation
    - Cholestasis-inducing medications, including but not limited to erythromycin, amoxicillin-clavulanate, angiotensin-converting enzyme (ACE) inhibitors, or terbinafine, or prochlorperazine (Prochlorperazine USPI)
21. Inability to tolerate antidiarrheal medications
22. Previous use of an ileal bile acid transporter inhibitor

Other Exclusions

23. Breastfeeding at the time of the screening visit or planning to breastfeed at any time starting with the screening visit through end of the study treatment period
24. Known intolerance/hypersensitivity to volixibat or its components
25. Confirmed positive urine drug/alcohol screen at any time during pregnancy, including at the screening visit
26. Participating in another ongoing interventional clinical study at screening or planning to participate in another contemporaneous interventional clinical study while participating in this study; contemporaneous participation in studies that do not involve pharmaceuticals, devices, or other interventions and do not interfere with this study's (OHANA) schedule of activities (i.e., observational studies, registries) are permitted
27. History of nonadherence to medical regimens, unreliability, medical condition, mental instability, or cognitive impairment that, in the opinion of the investigator or medical monitor, may interfere with the interpretation of study results, could compromise the validity of informed consent, compromise the safety of the participant, or lead to nonadherence with the study protocol or inability to conduct the study procedures
28. Participant is in a dependent relationship with or has an immediate family member who is a study site employee that is involved in the conduct of this study (e.g., spouse, parent, child, or sibling) or participant is a study site employee.

Investigational Product, Dosage, and Mode of Administration

Volixibat in capsules at doses of 20 mg and 80 mg and matched placebo will be supplied (in a blinded fashion for Part 2) for oral BID dosing. As much as possible, the morning dose (4 capsules) will be administered ~30 minutes before breakfast and the evening dose (4 capsules) will be administered 30 minutes before dinner to cover the luminal bile acid release associated with meals.

Statistical Methods

All predefined statistical analyses for the final analysis will be performed after the database is locked. All statistical analyses will be performed using SAS software (SAS Institute, Cary, NC, USA) version 9.4 or higher.

Primary Analysis for Primary Efficacy Endpoints

The primary analysis on the primary efficacy endpoint will be conducted in Part 2 using the modified intent-to-treat (mITT) population. A restricted maximum likelihood (REML)–based mixed-effects model for repeated measures (MMRM) will be used as the primary analysis method. The repeated measures include weekly postbaseline visits through Week 3, with the change from baseline in total sBA as the dependent variable. The MMRM model will include the fixed, continuous effects of baseline sBA and gestational age, fixed categorical effects of presence/absence of gestational diabetes, treatment group, visit, and treatment group-by-visit interaction and participant as a random effect.

The primary efficacy analysis will compare volixibat and placebo using the contrast (difference in least squares [LS] means) between treatment groups at Week 3. Significance tests will be based on LS means using a 2-sided significance level (2-sided 95% CIs).

The null hypothesis for the primary efficacy endpoint of the equality of volixibat and placebo is:

H_0: mean change in total sBA baseline and Week 3 in the 2 treatment groups are equal

Separately, a similar analysis on each of the open-label cohorts will be performed. The analysis will be identical, except that baseline sBA will not be a covariate used within the model.

Secondary Efficacy Endpoint Analyses

The secondary efficacy endpoints are designed to evaluate the efficacy of volixibat versus placebo for the treatment of pruritus in participants with ICP as well as composite perinatal outcomes.

The secondary efficacy endpoint for pruritus is defined as the mean change from baseline to Week 3 in the weekly average worst daily itch score as measured by the Adult ItchRO. The worst daily itch score is averaged over the 7 days preceding baseline and over the 7 days preceding each week through Week 3. If >3 days of assessments are missing from a given week, then that weekly average will be set to missing. The analysis of the Adult ItchRO will use an MMRM with a comparable model to that for the primary endpoint. The primary comparison will be made regardless of adherence to randomized treatment, treatment modification, or treatment termination and irrespective of any changes in, or initiations of, allowed concomitant therapies, while assuming prohibited medications are not available and that delivery or perinatal death prior to 3 weeks does not occur.

The attributes for this estimand are the same as for the primary estimand except for the below:

- Endpoint: Change from baseline to Week 3 of the study treatment period in the weekly averaged worse daily itch score as measured by the Adult ItchRO (average of the 7 days preceding baseline and of the 7 days preceding Week 3).
- Population-level summary: Difference in the mean change in the weekly averaged worse daily itch score

The secondary efficacy endpoint for composite perinatal outcomes is defined as the proportion of participants that experience any of the 4 outcomes specified in the protocol. The comparison of interest is

the proportion of participants meeting the composite outcome. The primary comparison will be made regardless of adherence to randomized treatment, treatment modification, or treatment termination and irrespective of any changes in, or initiations of, allowed concomitant therapies, while assuming prohibited medications are not available.

The attributes for this estimand are the same as for the primary estimand except for the below:

- Treatments: The randomized treatment administered BID until day of delivery (with at least 1 dose), regardless of adherence to randomized treatment, treatment modification, or treatment termination.
- Endpoint: Experiencing one or more of perinatal death, spontaneous preterm delivery, iatrogenic preterm delivery attributed to ICP or ICP-related complications, neonatal unit admission for ≥12 hours from birth until hospital discharge for one or more of predefined indications.
- Population-level summary: Difference between treatment groups in the proportion of participants achieving the endpoint.
- Intercurrent events: Preterm delivery or perinatal death are included as part of the endpoint definition and thus handled using a composite strategy.

For this responder-type endpoint, the number and proportion of participants that are considered a "responder" will be summarized by treatment group at Week 3 and analyzed using a stratified Cochran-Mantel-Haenszel (CMH) test to calculate the p-value for the difference between treatment groups. The p-value from the CMH test will be the basis of the hypothesis testing. Furthermore, as supportive analyses, a Logistic Regression (LR) estimator approach, as well as a logistic regression model with baseline sBA, gestational age, and presence/absence of gestational diabetes as covariates will be run.

All tests will be performed as 2-sided tests at the 0.05 level of significance.

Safety Analyses

All safety analyses will be performed on the safety population, defined as all participants who were randomized and received at least 1 dose of study medication.

For all safety and tolerability analyses, participants will be analyzed by the treatment received and the overall active treatment group. For active treatment, the dose received will be used.

Safety data collected at the baseline visit or the last preceding visit (if not collected at the baseline visit) will be used as the baseline value for safety analyses.

Safety measures include AEs, clinical laboratory values, physical examination findings (including body weight, height, and body mass index), vital signs, and concomitant treatment usage. For quantitative variables, descriptive statistics including number of observations, mean, median, standard deviation, minimum, and maximum will be presented for observed and change from baseline values at each study visit. Qualitative variables will be summarized using counts and percentages.

Interim Analysis

A formal interim analysis will be conducted at the end of Part 1, which will inform sample size for Part 2 as well as dose selection. There will be ongoing data review during Part 1 after every ~20 participants are enrolled in this open-label setting. Data from Part 1 will be included in the overall safety and tolerability summaries but will not be combined with those from Part 2 for the statistical inference performed in Part 2.

Sample Size Determination

Part 1 of the study is designed for estimation without formal statistical testing; hence, there are no formal power considerations. For example, with 20 participants per group and assuming a standard deviation of 25 for each group, a 95% CI for the mean for each volixibat arm would have a half width of 11.

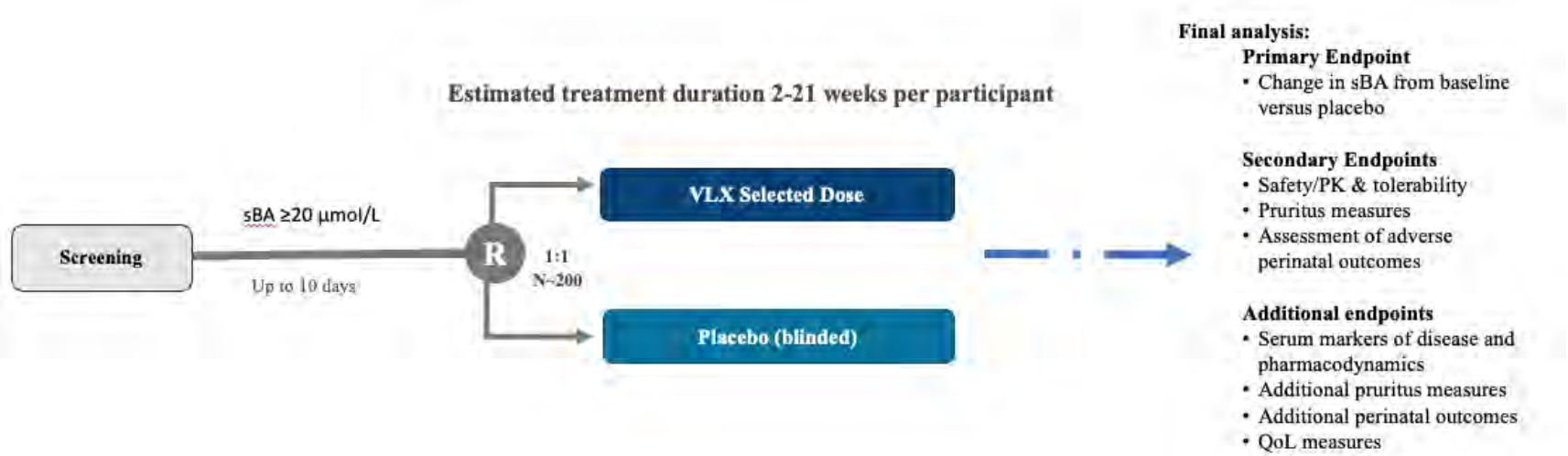
For the power calculations for Part 2, it is assumed that only a single dose will be selected at the end of Part 1, and a 2-sided alpha 0.05 will be used to test whether there is a difference between the 2 groups. Thus, for Part 2, a sample size of 100 participants per arm has 80% power to detect mean difference of 10 with standard deviation of 25. Power was calculated using SAS, version 9.4.

1.2. Study Schema

Figure 1 Part 1: Open Label (Proof of Concept)

BID=twice daily; PK=pharmacokinetic; R=randomization; sBA=serum bile acid; VLX=volixibat.

Figure 2 Part 2: Double Blind (Confirmatory Analysis)



PK=pharmacokinetic; QoL=quality of life; R=randomization; sBA=serum bile acid; VLX=volixibat.

1.3. Schedule of Activities

Table 1 Schedule of Activities

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	Day –10 to –1	0*	Every 7 Days (±1 Day) Until Delivery		Day of Delivery (+2 Days)	Phone Call: 14 Days after Delivery (±3 Days)	Clinic Visit: 28 Days after Delivery (±3 Days)	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
Administrative Procedures								
Informed consent	X							
Eligibility Procedures								
Inclusion/exclusion criteria	X	X						
Demography	X							
Prior/concomitant treatment	X	X	X	X	X	X	X	
Medical history	X	X						Includes menstrual and obstetric history
Estimated gestational age assessment	X							Confirmation of eligibility based upon estimated gestational age required before randomization: CRL from early ultrasound or head circumference on ultrasound if CRL is >84 mm.

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	**Day –10 to –1**	**0***	**Every 7 Days (±1 Day) Until Delivery**		**Day of Delivery (+2 Days)**	**Phone Call: 14 Days after Delivery (±3 Days)**	**Clinic Visit: 28 Days after Delivery (±3 Days)**	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
Hepatitis and HIV screen, TSH	X							If HIV results are documented as negative from the current pregnancy in a participant deemed to be low risk by the investigator, HIV at screening visit may be waived.
Dosing Procedures								
Randomization		X						
Study drug dispensing		X	X					As needed
Study drug collection/ reconciliation			X	X	X			As needed
Efficacy Procedures								
Education and compliance assessment of pruritus assessments	X	X	X					Education regarding importance of eDiary compliance at screening visit; reinforcement regarding eDiary compliance at study visits (as needed).
Once daily Adult ItchRO score assessment	X	X	X	X	X			Participant completes ItchRO at home on eDiary up to and including the day of delivery.

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	**Day –10 to –1**	**0***	**Every 7 Days (±1 Day) Until Delivery**		**Day of Delivery (+2 Days)**	**Phone Call: 14 Days after Delivery (±3 Days)**	**Clinic Visit: 28 Days after Delivery (±3 Days)**	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
Pruritus/QoL PRO assessments (5D itch scale, EQ-5D-5L, PROMIS SF v1.0-Fatigue 7a, PROMIS SF v1.0 - Sleep Disturbance, PIS-Itch)	X*	X**	X***	X	X****			Participant completes at clinic. All PRO assessments to be completed by participant, including PIS-Itch and PGIC-Itch, should be collected prior to additional procedures at study visits. *Participant to complete paper PIS-Itch at screening. This score will be captured in the EDC by site staff. ** Participant may complete all the PRO assessments at home before they arrive onsite for the baseline visit (Day 0). *** At Week 3 visit only **** For participants who terminate from study drug early, this does not need to be repeated.
PGIC-Itch			X*	X	X**			Participant completes at clinic. *At Week 3 visit only **For participants who terminate from study drug early, this does not need to be repeated.

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	**Day –10 to –1**	**0***	**Every 7 Days (±1 Day) Until Delivery**		**Day of Delivery (+2 Days)**	**Phone Call: 14 Days after Delivery (±3 Days)**	**Clinic Visit: 28 Days after Delivery (±3 Days)**	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
CSS		X	X*	X	X		X	To the extent possible, CSS assessments should be made by the same investigator or designee during study visits. *At Week 3 visit only.
Other Procedures								
Health utilization		X		X			X	
Safety Procedures								
Drug and alcohol screen	X							
Physical examination	X	X	X	X	X		X	Complete examination conducted at screening and F2 visits. A symptom-directed examination focused on changes from last examination conducted at other visits. Includes confirmation of fetal cardiac activity via Doppler/auscultation at each visit while participant is pregnant.
Height	X							
Weight	X	X	X	X	X		X	

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	**Day –10 to –1**	**0***	**Every 7 Days (±1 Day) Until Delivery**		**Day of Delivery (+2 Days)**	**Phone Call: 14 Days after Delivery (±3 Days)**	**Clinic Visit: 28 Days after Delivery (±3 Days)**	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
ECG	X							ECG is not a required study procedure; however, a local ECG at or after screening visit may be performed at the investigator's discretion.
Vital signs	X	X	X	X	X		X	
Hematology	X		X	X	X		X	
Chemistry	X		X	X	X		X	
Coagulation panel (PT/INR, PTT)	X		X	X	X		X	
Vitamin A, D, and E levels		X	X*		X		X	*Every 4 weeks, starting with Week 4 visit. The sample collection tube should be protected from light.
Urinalysis	X	X	X	X	X		X	
Lipid panel, glycated albumin	X		X*		X		X	*Every 2 weeks, starting with Week 2 visit
AE review		X	X	X	X	X	X	AEs for mother and baby to be collected and reviewed for up to 30 days after respective dates of hospital discharge. See Section 8.3.1 for further details.

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	**Day –10 to –1**	**0***	**Every 7 Days (±1 Day) Until Delivery**		**Day of Delivery (+2 Days)**	**Phone Call: 14 Days after Delivery (±3 Days)**	**Clinic Visit: 28 Days after Delivery (±3 Days)**	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
Fetal ultrasound	X*	X*	X**					* First ultrasound may be done during screening OR baseline (both are not required). The first study ultrasound (screening or baseline) may be waived if an ultrasound was already performed within 1 week prior to screening and should be captured in the eCRF on the screening ultrasound page. ** Every 4 weeks while on study drug
Antepartum fetal monitoring (NST/CTG or BPP)		X						At baseline visit only, and thereafter at the discretion of the investigator

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	**Day –10 to –1**	**0***	**Every 7 Days (±1 Day) Until Delivery**		**Day of Delivery (+2 Days)**	**Phone Call: 14 Days after Delivery (±3 Days)**	**Clinic Visit: 28 Days after Delivery (±3 Days)**	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
Perinatal outcomes assessment					X			Outcomes include • Gestational age at delivery • Mode of delivery (e.g., vaginal, operative vaginal, Cesarean, etc.; and iatrogenic vs. spontaneous) • Indication(s) for delivery, if iatrogenic • Maternal estimated blood loss at delivery • Apgar score at 5 minutes • Infant/placenta weight • Presence/absence of meconium staining of amniotic fluid • Neonatal diagnoses and data, including NICU data if applicable
Umbilical cord sample (at delivery, when possible)					X			Umbilical artery pH should be assessed first, followed by volixibat pharmacokinetics. Thereafter, sBA, total cholesterol, LDL cholesterol, and glucose can be assessed in any order.
Genetics – maternal blood (optional)		X						Specific testing TBD. Must confirm specific consent has been obtained for genetic testing

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	**Day –10 to –1**	**0***	**Every 7 Days (±1 Day) Until Delivery**		**Day of Delivery (+2 Days)**	**Phone Call: 14 Days after Delivery (±3 Days)**	**Clinic Visit: 28 Days after Delivery (±3 Days)**	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
Genetics – cord blood sample (optional)					X			Specific testing TBD. Must confirm specific consent has been obtained for genetic testing.
NICU admission					X	X	X	Collect NICU admissions diagnoses and data for the duration of NICU stay (i.e., follow to resolution).
Drug Levels/Biomarkers Procedures								
Volixibat concentration sample (maternal plasma)			X*	X	X			*At Weeks 1 and 3 visit, 3 hours (±1 hour) post-dose When feasible, maternal sample should be collected as close to time of actual delivery as possible. Drug level sampling may be discontinued in Part 2 pending Part 1 results
Volixibat concentration sample (fetal plasma from umbilical cord at delivery, when possible)					X			Drug level sampling may be discontinued in Part 2 pending Part 1 results

Visit Name	Screening	Treatment Period				Follow-Up		Notes
		Baseline	Weeks 1, 2, 3, etc.	Early Termination*	EOT/Delivery	F1	F2 (EOS)	*Participants who early terminate from study drug should still continue with weekly visits through delivery, F1, and F2.
Day/Week (Window)	**Day –10 to –1**	**0***	**Every 7 Days (±1 Day) Until Delivery**		**Day of Delivery (+2 Days)**	**Phone Call: 14 Days after Delivery (±3 Days)**	**Clinic Visit: 28 Days after Delivery (±3 Days)**	Some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations. *See Section 8.1.8 for further details on timing of baseline visit.
sBA	X	X*	X*	X	X**		X	At each visit except EOT/Delivery, sBA should be drawn 30-90 minutes postprandially, whenever possible). Every effort should be made to collect sBA levels at approximately the same time of day (morning, midday, or afternoon) at each visit starting with baseline, except for EOT/delivery. *At baseline and Week 3, there will be 2 samples collected. **At EOT/Delivery, there will be 2 samples collected, without regard to last meal.
Postprandial biomarker samples (maternal) • 7αC4 (postprandial, 30-90 minutes) • FGF-19 • Autotaxin • Progesterone sulfate • GLP-1		X	X*		X			Prior to the sample collection, all participants are strongly encouraged to eat a meal within approximately 30-90 minutes (whenever possible). Every effort should be made to collect biomarker sample collection at approximately the same time of day (morning, midday, or afternoon) at each visit. *At Week 3 visit only

5-D Itch; Adult Itch RO; 7αC4=7α-hydroxy-cholesten-3-one; AE=adverse event; BPP=biophysical profile; CRL=crown rump length; CSS=Clinician Scratch Scale; CTG=cardiotocography; eCRF=electronic case report form; eDiary=electronic diary; EDC=electronic data capture; EOS=end of study; EOT=end of treatment;

EQ-5D-5L=EuroQoL 5-Dimension 5-Level; ET=early termination; F1=Follow-Up Visit 1; F2=Follow-Up Visit 2; INR=international normalized ratio; ItchRO=Itch Reported Outcome; LDL=low-density lipoprotein; NICU=neonatal intensive care unit; NST=nonstress test; PGIC-Itch=Patient Global Impression of Change-Itch; PIS-Itch=Patient Impression of Severity-Itch; PRO=patient-reported outcome; PROMIS=Patient-Reported Outcomes Measurement Information System; PT=prothrombin time; PTT=partial thromboplastin time; QoL=quality of life; sBA=serum bile acid; SF=short form; TBD=to be determined; TSH=thyroid-stimulating hormone.

LIST OF ABBREVIATIONS

Abbreviation	Definition
7αC4	7α-hydroxy-cholesten-3-one
ACE	angiotensin-converting enzyme
AE	adverse event
AESI	adverse event of special interest
ANCOVA	analysis of covariance
BID	twice daily
BMI	body mass index
BPP	biophysical profile
C_{max}	maximum observed concentration
CMH	Cochran Mantel Haenszel
CRL	crown-rump length
CRO	contract research organization
CSS	Clinician Scratch Scale
CTCAE	Common Terminology Criteria for Adverse Events
CTG	cardiotocography
DART	developmental and reproductive toxicology
DILI	drug-induced liver injury
ECI	event of clinical interest
eCRF	electronic case report form
eDiary	electronic diary
EQ-5D-5L	EuroQol 5-dimension 5-level
F2	follow-up Visit 2
fBA	fecal bile acid
GCP	Good Clinical Practice
GI	gastrointestinal
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
IB	Investigator's Brochure
IBAT	ileal bile acid transporter
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICP	intrahepatic cholestasis of pregnancy

Abbreviation	Definition
IDMC	independent data monitoring committee
IEC	independent ethics committee
INR	international normalized ratio
IRB	institutional review board
IRT	interactive response technology
ItchRO	Itch Reported Outcome
IV	intravenous
JAK	Janus kinase
LDL	low-density lipoprotein
LLOQ	lower limit of quantitation
LS	least squares
MAR	missing at random
medical monitor	sponsor or CRO medical monitor
MI	multiple imputation
mITT	modified intent-to-treat
MMRM	mixed-effects model for repeated measures
MNAR	missing not at random
NASH	nonalcoholic steatohepatitis
NICU	neonatal intensive care unit
NRS	numerical rating scale
NST	nonstress test
OR	odds ratio
PBC	primary biliary cirrhosis
PD	pharmacodynamic
PFIC	progressive familial intrahepatic cholestasis
PGIC-Itch	Patient Global Impression of Change-Itch
PIS-Itch	Patient Impression of Severity-Itch
PK	pharmacokinetic
PPAR	peroxisome proliferator-activated receptor
PPROM	preterm premature rupture of the membranes
PRO	patient-reported outcome
PROMIS®	Patient-Reported Outcomes Measurement Information System
PSC	primary sclerosing cholangitis
QD	once daily
QTcF	QT interval corrected using Fridericia's correction formula

Abbreviation	Definition
RCOG	Royal College of Obstetricians and Gynaecologists
SAE	serious adverse event
SAP	statistical analysis plan
sBA	serum bile acid
SF	Short Form
SoA	schedule of activities
SOC	System Organ Class
TEAE	treatment-emergent adverse event
T_{max}	time to reach C_{max}
UDCA	ursodeoxycholic acid
WI-NRS	Worst Itch Numeric Rating Scale

2. INTRODUCTION

2.1. Background on Intrahepatic Cholestasis of Pregnancy

Intrahepatic cholestasis of pregnancy (ICP) is a rare cholestatic disorder that occurs during pregnancy and is associated with maternal changes in liver function, elevated levels of serum bile acids (sBAs), and intractable pruritus, as well as adverse perinatal outcomes (EASL 2009; Geenes and Williamson 2009; Williamson and Geenes 2014; Ozkan et al. 2015; Wood et al. 2018). Cholestasis refers to an impairment of bile secretion and/or bile flow, which may clinically present with symptoms such as pruritus, fatigue, and jaundice and may be associated with changes in liver biochemistry markers. Intrahepatic cholestasis is defined as cholestasis resulting from hepatocellular functional defects or obstructive lesions of the intrahepatic biliary tract distal from bile canaliculi.

ICP is characterized by pruritus, which is frequently intense and intractable, with typical onset in the second or third trimester of pregnancy and elevated serum aminotransferases and sBA levels. ICP is also frequently associated with an abnormal maternal metabolic profile. In addition to the hepatic features, there is an increased prevalence of dyslipidemia and impaired glucose tolerance in patients with ICP (Martineau et al. 2015). Gestational diabetes and preeclampsia also occur more commonly in women with ICP (Goulis et al. 2004; Martineau et al. 2014). After delivery, there is rapid (within a few weeks) resolution of signs and symptoms.

ICP is also associated with an increased risk for adverse perinatal outcomes, including preterm birth and life-threatening or even fatal consequences for the fetus, such as stillbirth (Sepulveda et al. 1991; Williamson et al. 2001; Ovadia et al. 2019).

Although ICP resolves rapidly following delivery, it is associated with an increased risk of future morbidity, including higher incidence of hepatobiliary diseases such as hepatitis, fibrosis, and gallstones and increased risk of liver and biliary tree cancer, immune-mediated disease, and cardiovascular disease. Moreover, oral contraceptives, in particular those containing estrogens, may cause recurrence of symptoms, and guidelines recommend the use of alternative methods of contraception (Bicocca et al. 2018). Furthermore, patients with ICP can suffer from jaundice or present with vitamin K deficiency, which can lead to prolonged coagulation time and increase the risk of intrapartum and postpartum hemorrhage (Mohan et al. 2019).

Generally speaking, ICP is diagnosed if sBA levels are approximately ≥10 µmol/L in a pregnant woman with new onset of pruritus during pregnancy and no other known pathology thought to be responsible for the laboratory findings and/or symptoms (Bicocca et al. 2018). ICP can be classified as mild or severe depending on the level of accumulation of sBA, with 40 µmol/L often used as the threshold value that is considered severe (Bicocca et al. 2018).

2.2. Study Rationale

The pathophysiology of ICP in the mother is incompletely understood; however, there is extensive clinical evidence that elevated maternal sBA levels are linked to maternal and fetal morbidity and mortality associated with ICP, suggesting that elevated maternal sBA plays a causal role in these adverse clinical outcomes (Sepulveda et al. 1991; Williamson et al. 2001; Glantz et al. 2004; Geenes et al. 2014; Mohan et al. 2019; Ovadia et al. 2019).

The pathophysiology of adverse perinatal outcomes in ICP is likely related to the elevated sBA levels in the expectant mother (Ovadia et al. 2019). In pregnancies uncomplicated by ICP, a physiologic sBA gradient across the placenta facilitates the disposal of fetal bile acids into the maternal blood stream, with ultimate elimination of both maternal and fetal bile acids via maternal feces (Monte et al. 1995). However, in ICP, elevated sBA levels on the maternal side cause a reduction or reversal of the physiologic transplacental gradient, resulting in the accumulation of bile acids in the fetal compartment, as evidenced by the detection of elevated levels in amniotic fluid, cord serum, and meconium (Shaw et al. 1982; Geenes and Williamson 2009; Williamson and Geenes 2014). Increased bile acid concentrations are associated with toxic effects, such as fetal cardiac arrhythmia and placental vessel spasms (Sepulveda et al. 1991; Williamson et al. 2001). Such effects can have potentially life-threatening or even fatal consequences for the fetus, and data from in vitro and animal studies support the role of elevated levels of bile acids in the promotion of preterm labor and the cause of the presence of meconium in the amniotic fluid, neonatal respiratory distress, and stillbirth (Geenes et al. 2014; Williamson and Geenes 2014). Several cohort studies as well as recent systematic reviews with meta-analyses have clearly demonstrated the increased risk of adverse perinatal outcomes in ICP and their correlation with maternal sBA levels (Glantz et al. 2004; Geenes et al. 2014; Mohan et al. 2019; Ovadia et al. 2019):

- The risk of stillbirth has been shown to increase in women with ICP who have sBA concentrations >100 μmol/L and increases as gestation progresses (Ovadia et al. 2019), suggesting a likely benefit from reduction of sBA promptly upon detection of elevated levels.
- In a large prospective population-based case-control study performed in the United Kingdom, which included 669 women with severe ICP (sBA ≥40 μmol/L at any time during pregnancy) and a singleton pregnancy, a doubling in sBA level increased the risk of all preterm delivery by 68%, of spontaneous preterm delivery by 66%, of meconium staining of the amniotic fluid by 55%, and of stillbirth by 200% (Geenes et al. 2014).

Increased bile acid concentrations appear to play a primary role in the development of the characteristic cholestatic pruritus associated with ICP, which is typically clinically significant, and in some cases is an indication to iatrogenic preterm delivery (Chappell et al. 2019). The link is complicated by how specific hydrophilic and hydrophobic subspecies may interplay in pruritus. It is possible, however, that a certain profile of sBA and/or pruritogen(s) is necessary to mediate pruritus (Bergasa 2014). However, accumulation of bile acid as a result of cholestatic liver disease, including ICP, has been associated with pruritus, although direct correlation of sBA levels to severity of itch is debatable (Pathak et al. 2010; Kremer et al. 2012). This association of sBA and pruritus is further supported by evidence from pregnancies in other cholestatic conditions. In pregnant women with non-ICP–induced intrahepatic or extrahepatic cholestasis, such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), respectively, in which systemic bile acid concentrations are typically elevated, similar maternal and fetal morbidity and mortality are observed (Efe et al. 2014; Patel et al. 2019). Based on the collective data, the elevation of sBA plays a direct causal role on the characteristic maternal and fetal pathology of ICP.

2.2.1. Overview of Current Therapeutic Interventions and Rationale for Use of Volixibat in ICP

Treatment of ICP is directed toward 2 main goals: reducing symptoms in the expectant mother, in particular cholestatic pruritus, and reducing adverse perinatal outcomes.

Off-label use of ursodeoxycholic acid (UDCA; except in France where it is approved for the treatment of ICP) has been the only option consistently proposed in guidelines as a treatment option for ICP. However, evidence is mixed regarding its benefit. Two Cochrane systematic reviews of the effectiveness of UDCA for ICP have concluded that it might ameliorate pruritus to a small extent but that definitive evidence for improvement in perinatal outcomes was absent (Gurung et al. 2013; Walker et al. 2020). Additionally, a recent meta-analysis of 13 studies also concluded that UDCA did not significantly improve maternal or fetal outcomes (Shen et al. 2019). In a recent double-blind, multicenter, randomized placebo-controlled study that enrolled 605 women with ICP in the United Kingdom (Chappell et al. 2019), UDCA was not effective in reducing a composite of adverse perinatal outcomes, defined as in utero fetal death or neonatal death, preterm delivery, or neonatal unit admission. Although UDCA appeared to be safe, it had no clinically meaningful effect on maternal itch symptoms. It did not reduce maternal sBA concentrations, and the reduction in ALT was of uncertain clinical significance. In contrast to the randomized, placebo-controlled data from PITCHES, a recent individual patient data meta-analysis suggested that UDCA treatment in patients with ICP was associated with a statistically significant reduction in spontaneous preterm births in singleton pregnancies with initial sBA levels ≥40 μmol/L without a significant reduction in stillbirth, compared with no UDCA treatment (Ovadia et al. 2021). Notably, the patients not treated with UDCA were treated with placebo, S-adenosyl-methionine, and/or other off-label therapies for ICP.

In another small survey-based study, rifampicin use in combination with UDCA was associated with lower sBA levels in women with ICP (Geenes et al. 2015). However, rifampicin treatment can be associated with hepatotoxicity, which affected up to 13% of participants in a study of PBC (Bachs et al. 1992), and the potential fetal side effects are unknown.

Besides UDCA and rifampicin, cholestyramine, dexamethasone, and S-adenosyl-L-methionine have demonstrated some evidence of maternal benefit in ICP. However, none of the aforementioned therapeutic options is considered as first-line treatment (EASL 2009; Tran et al. 2016; Bicocca et al. 2018). Vitamin K supplementation is also frequently used in cases of vitamin K deficiency to limit the risk of intrapartum and postpartum hemorrhage.

Temporary relief from pruritus may be achieved in some women through the use of over-the-counter topical treatments or antihistamines. However, such therapeutic options provide limited and inconsistent relief and do not address the underlying pathologic mechanism of pruritus and the resulting potentially serious or fatal consequences for the fetus.

In summary, UDCA use has shown limited and conflicting supporting evidence. As a result, patients with ICP often cycle through UDCA and off-label therapies without relief, which may result in a decision to induce delivery preterm, increasing the risk of preterm-associated morbidity and mortality to the fetus (Chappell et al. 2019).

Although recommendations are inconsistent across guidelines and the practice is not evidence based, active management of pregnancy by early elective delivery between 36 and 38 weeks is performed on a case-by-case basis in some regions of the world and seems to have been

successful at reducing the intrauterine death rate (EASL 2009; Tran et al. 2016; Bicocca et al. 2018; ACOG 2019). For example, a recent guidance from the Society for Maternal-Fetal Medicine (Lee et al. 2021) recommends elective delivery for patients with ICP and total sBA levels of <100 µmol/L between 36 weeks 0 days and 39 weeks 0 days of gestation; patients with total sBA levels ≥100 µmol/L should be offered delivery at 36 weeks of gestation. This practice is associated with its own risks for the baby, ranging from short-term neonatal problems to long-term issues such as cerebral palsy; disorders of psychological development, behavior, and emotion; and other major disabilities such as impaired vision and hearing (Moster et al. 2008; Saigal and Doyle 2008; MacKay et al. 2010; Quigley et al. 2012).

In summary, although symptoms are transient for the mother, ICP is associated with increased maternal morbidity, increased risk of life-threatening or even fatal sequelae for the fetus, and chronically debilitating consequences for surviving infants. This is reflected in the widespread policies to manage severe cases of ICP by elective early delivery between 36- and 38-weeks' gestational age or earlier to reduce the risk of late stillbirth and alleviate maternal symptoms. This practice is not evidence based (EASL 2009; Tran et al. 2016; Bicocca et al. 2018; ACOG 2019) and is associated with potentially severe and/or chronic sequela.

The current study is designed to assess the potential of volixibat to reduce maternal pruritus and fetal morbidity and mortality associated with ICP, which would provide a significant efficacy benefit over currently utilized therapies. In ICP, lowering of (or mitigating potential further increases in) maternal sBA would be expected to ameliorate the increased risk of adverse perinatal outcomes associated with increased sBA levels by promoting restoration of the physiologic transplacental gradient and alleviating the accumulation of toxic bile acids in the fetus. Given the evidence supporting the role of elevated sBA as a driver of adverse perinatal outcomes, the mean change in total maternal sBA concentration has been selected as the primary endpoint as a surrogate for clinically relevant perinatal outcomes. Perinatal outcomes will also be directly assessed using a composite measure as a key secondary efficacy endpoint.

The current study design is divided into 2 parts. Part 1 is an open-label phase that provides real-time assessments of safety/tolerability of volixibat 20 mg and 80 mg as well as data for dose exploration and selection for Part 2 of the study. Part 2 is a randomized, double-blind, placebo-controlled phase for confirmatory analysis comparing the selected volixibat dose with placebo for superior efficacy.

2.3. Volixibat Pharmaceutical and Therapeutic Background

Volixibat is an inhibitor of the ileal bile acid transporter (IBAT). Inhibition of IBAT interrupts normal enterohepatic recirculation of bile acids, significantly increasing the amount of bile acid excreted in feces in treated participants and lowering circulating bile acid levels. Bile acids and their recirculation are understood to be at the center of cholestatic pruritus pathophysiology, and IBAT inhibitors similar to volixibat, such as maralixibat and GSK2330672, have demonstrated efficacy in pruritus and sBA reductions associated with progressive familial intrahepatic cholestasis (PFIC), Alagille syndrome, PBC, and PSC (Hegade et al. 2017; Thompson et al. 2019; Bowlus et al. 2019; Gonzales et al. 2019a, 2019b; Mayo et al. 2019). In nonclinical and clinical studies, treatment with volixibat has been shown to affect key pharmacodynamic (PD) parameters associated with IBAT inhibition, similar to other IBAT inhibitors. These include increased fecal bile acid (fBA) excretion, in conjunction with increases

in 7α-hydroxy-cholesten-3-one (7αC4) and decreases in low-density lipoprotein (LDL) cholesterol; both are PD measures indicating increased bile acid synthesis to compensate for increased bile acid excretion and a reduction in the bile acid pool in the body caused by effective IBAT inhibition.

UDCA is used as a potential treatment for ICP despite evidence of limited efficacy (see Section 2.2.1). The exact mechanism by which UDCA could provide benefit in ICP and other cholestatic diseases is not understood but could be due to its relatively hydrophilic nature compared with other bile acids. While volixibat inhibits the recirculation of bile acids, it is important to note that no drug-drug pharmacokinetic (PK) interaction is expected between volixibat and UDCA. The preferred substrates for IBAT are conjugated bile acids. Since UDCA is a non-conjugated bile acid likely absorbed through passive diffusion, IBAT inhibition is not predicted to have an effect on the bioavailability of oral UDCA. Published modeling of the pharmacokinetics of UDCA and its metabolites in the presence and absence of IBAT inhibition predicts the absence of a PK interaction (Zuo et al. 2016). In addition, clinical UDCA PK data in the context of an IBAT inhibitor dosed in patients with PBC showed no drug-drug interaction between UDCA and the IBAT inhibitor (Hegade et al. 2017).

A detailed description of the chemistry, pharmacology, efficacy, and safety of volixibat is provided in the Investigator Brochure (IB).

2.4. Key Nonclinical and Clinical Studies

This is the first study evaluating volixibat for the treatment of participants with ICP. An extensive nonclinical PK; absorption, distribution, metabolism, and excretion (ADME); and safety package has been completed on volixibat, including a full battery of safety pharmacology studies, 4 different types of genotoxicity tests, and chronic general toxicity testing in rats (up to 26-week duration) and in dogs (up to 39-week duration). To prepare for the proposed ICP clinical study in the more vulnerable population of pregnant women, the battery of developmental and reproductive toxicology (DART) testing was also expanded, including fertility and early embryonic development testing in rats, embryo/fetal development testing in rats and rabbits, and prenatal and postnatal development testing (including maternal function) in rats. No concerning toxicities were observed in any of these studies, and very large safety margins (14-160X depending on animal study, under the assumption of a human dose of 80 mg twice daily [BID]) were established between the animal doses and the highest proposed human dose.

Volixibat is minimally absorbed after oral dosing at all doses tested. The highest plasma concentration that has been recorded in previous clinical studies is 0.64 ng/mL (0.79 nM). Food does not affect the absorption of volixibat. Elimination is via fecal excretion with negligible metabolism and urinary excretion (70% eliminated in the feces within 24 hours, >90% from feces over the full course of a clinical mass balance study). Metabolism and urinary excretion are negligible.

Volixibat administration reduces systemic bile acids in all populations and species studied as evidenced by rapid and significant fecal excretion and consistent changes in markers indicative of an interruption of enterohepatic recirculation bile acids across healthy volunteers and participants with nonalcoholic steatohepatitis (NASH). In addition, in instances where

participants demonstrated elevated baseline levels of sBA, rapid and significant decreases in sBA were observed on volixibat treatment, consistent with the significant decreases in sBA observed in a nonclinical model of mild cholestasis. The consistent PD effects of volixibat observed on bile acids and serum 7αC4 in previous clinical studies indicate that volixibat inhibits bile acid reabsorption and supports the potential utility in treating patients with ICP.

Refer to the Volixibat IB for detailed information regarding nonclinical and clinical studies with volixibat.

2.5. Benefit/Risk Assessment

By virtue of volixibat's ability to inhibit IBAT-mediated bile acid reabsorption, there is an increase in bile acid excretion via the intestine and feces that results in lower bile acid levels in the serum. It is hypothesized that these net reductions in bile acid levels will, in turn, have a beneficial impact on pathological processes and symptoms in patients with diseases associated with elevated bile acid levels, such as ICP. Volixibat has been well tolerated in clinical studies to date with the most common adverse events (AEs) being mild and transient gastrointestinal (GI) symptoms. No clinically significant safety concerns were observed based on review of safety data across all participants exposed to volixibat.

As stated above, clinical data suggest extremely low systemic exposure to volixibat, because it is minimally absorbed with concentrations obtained in humans at the intended clinical doses being typically below the lower limit of quantitation (LLOQ; LLOQ=0.05 ng/mL). Taken together with the findings from the battery of DART testing, there is minimal risk of exposure to the fetus or harm to the fetus upon potential exposure to volixibat.

Based upon the above, the benefit-risk profile of volixibat in patients with ICP, as well as in their neonates, is predicted to be favorable. More detailed information about the known and expected benefits and risks, including the expected adverse reactions of volixibat, may be found in the Volixibat IB.

3. OBJECTIVES AND ENDPOINTS

The following objectives and endpoints will be evaluated in participants who meet study eligibility criteria.

3.1. Primary Objective and Endpoint

Part 1:

- To assess the safety and tolerability of volixibat in participants with ICP on the basis of the following endpoints:
    - Proportion of participants experiencing one or more of the following:
        - Treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), adverse events of special interest (AESIs), events of clinical interest (ECIs), and AEs that lead to discontinuation of study drug
        - Clinically significant laboratory abnormalities

Part 2:

- To assess the efficacy of volixibat on the reduction of elevated sBA concentrations in participants with ICP on the basis of the following endpoint:
  - Mean change from baseline to Week 3 of the treatment period in total sBA concentration

3.2. Secondary Objectives and Endpoints

The following secondary objectives and endpoints are applicable in Part 1 and Part 2.

- To assess the efficacy of volixibat on pruritus due to ICP on the basis of the following endpoint:
  - Mean change from baseline to Week 3 of the treatment period in the weekly average worst daily itch score as measured by the Adult Itch Reported Outcome (ItchRO)
- To assess the impact of volixibat on a composite perinatal outcome in participants with ICP on the basis of the following endpoints:
  - Proportion of participants experiencing one or more of the following:
    - Perinatal death, defined as in utero fetal death after randomization or known neonatal death up to 28 days after birth
    - Spontaneous preterm delivery, defined as delivery at <37 weeks' gestational age following spontaneous onset of preterm labor or preterm premature rupture of the membranes (PPROM)
    - Iatrogenic preterm delivery attributable to ICP or ICP-related complications, defined as delivery at <37 weeks' gestational age resulting from medical intervention (i.e., augmentation/induction of labor or cesarean delivery without prior spontaneous onset of preterm labor or PPROM with one or more of the following as the indication for delivery):
      - Diagnosis of ICP with or without suspected fetal compromise
      - Persistently elevated/increasing sBA
      - Worsening hepatic function
      - Intolerable maternal ICP symptoms
    - Neonatal unit admission for ≥12 hours from birth until hospital discharge for one or more of the following indications:
      - Respiratory insufficiency/failure with requirement for oxygen supplementation
      - Noninvasive or invasive respiratory support
      - Hemodynamic instability or shock requiring clinical intervention
      - Metabolic acidosis or signs of asphyxia

- Proven infection
- Hypoglycemia or feeding problems requiring intravenous (IV) fluids or tube feeding
- Meconium-stained amniotic fluid and/or its sequelae (e.g., meconium aspiration, etc.)

3.3. Safety Objective and Endpoints

- To assess the safety and tolerability of volixibat in participants with ICP on the basis of the following endpoints in Part 2:
  - Incidence of TEAEs, SAEs, AESIs, ECIs, and AEs that lead to discontinuation of study drug
  - Incidence of clinically significant laboratory abnormalities

3.4. Exploratory Objectives and Endpoints

The following exploratory objectives and endpoints are applicable to Part 1 and Part 2:

- To assess volixibat drug levels in maternal and fetal plasma and PD markers in maternal blood in participants with ICP on the basis of the following endpoints:
  - Volixibat concentrations in maternal and fetal plasma (from umbilical cord sampling)

    Note: Assessment of maternal/fetal volixibat drug levels is a secondary objective for Part 1.
  - Change in biomarkers of bile acid synthesis, inflammation, and pruritogens
  - Proportion of participants with sBA <40 μmol/L at end of the treatment period
- To assess the longer-term efficacy of volixibat in participants with ICP on the basis of the following endpoints:
  - Mean change from Week 3 to end of treatment period in total sBA concentration
  - Mean change from baseline to end of treatment period in total sBA concentration
  - Association between changes from baseline to Week 3 in total sBA and ItchRO as well as their association with the composite perinatal outcome
- To assess the effect of volixibat on additional perinatal outcomes on the basis of the following endpoints:
  - Mean gestational age at delivery
  - Proportion of participants with an early term delivery (37–38 weeks 6 days, inclusive)
  - Proportion of participants with a full-term delivery (≥39 weeks)
  - Proportion of participants experiencing a perinatal death
  - Proportion of participants with a spontaneous preterm delivery

  - Proportion of participants with an iatrogenic preterm delivery attributable to ICP
  - Proportion of neonates requiring neonatal intensive care unit (NICU) admission for ≥12 hours between birth and hospital discharge
  - Proportion of neonates with meconium-stained amniotic fluid at delivery
  - Proportion of participants requiring rescue medication for ICP
  - Proportion of neonates with Apgar score <7 at 5 minutes of life
  - Mean birth weight percentile
  - Mean placental weight percentile
  - Proportion of neonates with umbilical cord pH <7.0 at birth
  - Means for umbilical cord blood sBA, total cholesterol, LDL cholesterol, and glucose
  - Mean maternal estimated blood loss at delivery
  - Mean time from randomization to delivery
- To evaluate the effect of volixibat on additional measures of pruritus efficacy and quality of life in participants with ICP on the basis of the following endpoints:
  - Change from baseline in the 5-D Itch Scale
  - Change from baseline in Clinician Scratch Scale (CSS)
  - Change from baseline in EuroQoL 5-dimension, 5-level (EQ-5D-5L)
  - Change from baseline in Patient-Reported Outcomes Measurement Information System (PROMIS®) Short Form (SF) Fatigue 7a
  - Change from baseline in PROMIS SF Sleep Disturbance
  - Change from baseline in Patient Impression of Severity-Itch (PIS-Itch)
  - Patient Global Impression of Change-Itch (PGIC-Itch)
- To assess the impact of volixibat on healthcare utilization in participants with ICP on the basis of the following endpoints:
  - Number and duration of medical care encounters, including surgeries and other selected procedures (inpatient and outpatient)
  - Dates and duration of hospitalization (total days or length of stay, including duration by wards [e.g., intensive care unit])
  - Number and type of diagnostic and therapeutic tests and procedures
  - Outpatient medical encounters and treatments (including physician or emergency department visits, tests and procedures, and medications)

4. STUDY DESIGN

4.1. Overall Design

This is an operationally seamless adaptive clinical study that will consist of 2 parts. Part 1 of the study is open label. Part 2 of the study constitutes a randomized, double-blind, placebo-controlled phase comparing the selected volixibat dose with placebo for superior efficacy.

Part 1: Proof of Concept, Safety/Tolerability, Dose Ranging, Pharmacokinetics, and Interim Analysis

After a screening period of up to 10 days, eligible participants diagnosed with ICP with screening sBA ≥10 µmol/L will be randomized with stratification (based on background use of UDCA or not, on gestational age [<32 weeks or ≥32 weeks] at randomization, and presence/absence of gestational diabetes) in a 2-arm (1:1), open-label fashion to receive volixibat 20 mg BID or volixibat 80 mg BID.

Study-drug dosing will begin on Day 0 and continue until end of the treatment period, defined as the day of delivery. Study visits to assess safety, plasma concentrations of volixibat, pharmacodynamics, and efficacy will be conducted from baseline (Day 0) until delivery at time points specified in the schedule of activities (SoA; Table 1). Because alternative measures may be implemented when necessary for public health or other emergency situations, and when sufficient to help ensure the safety of study participants, some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or designee and in accordance with local and regional regulations (Appendix 11; FDA 2020). Participants will also enter ItchRO assessments into an electronic diary (eDiary) once daily (QD). Use of limited rescue therapies for ICP will be permitted for participants who meet protocol-specified criteria.

Interim analyses are detailed in Section 10.4.4.

Part 2: Confirmation of Safety and Efficacy with Selected Dose

For Part 2, it is anticipated that ~200 participants (100 per arm) will be randomized after the volixibat dose is selected in Part 1. Participants with ICP with a screening sBA level ≥20 µmol/L who also meet the ItchRO criteria (see Section 5.1) will be randomized with stratification (based on gestational age [<32 weeks or ≥32 weeks] at randomization, presence/absence of gestational diabetes, and highest sBA before randomization [<40 µmol/L, ≥40 µmol/L to <100 µmol/L, or ≥100 µmol/L]) in a 2-arm (1:1), double-blind fashion to receive the selected volixibat dose BID or placebo.

Study visits to assess safety, pharmacodynamics, efficacy, and plasma concentrations of volixibat in all participants will be conducted from baseline (Day 0) until delivery at time points specified in the SoA (Table 1); sampling for volixibat concentrations may be discontinued in Part 2 if Part 1 results demonstrate consistently negligible or below the limit of quantification concentrations in maternal and umbilical cord (fetal) plasma. Because alternative measures may be implemented, when necessary, for public health or other emergency situations and when sufficient to help ensure the safety of study participants, some visits may be conducted as a home health or remote visit at the discretion of the investigator upon approval by the sponsor or

designee and in accordance with local and regional regulations. Participants will also enter ItchRO assessments into an eDiary QD. Use of limited rescue therapies for ICP will be permitted for participants who meet protocol-specified criteria.

Safety

In both Part 1 and Part 2, safety assessments will include AEs, clinical laboratory tests, vital signs, and physical examinations. Additional fetal safety assessments will include serial ultrasounds to assess fetal growth and nonstress tests (NSTs)/cardiotocography (CTG) or fetal biophysical profile (BPP). Assessment of plasma concentrations of volixibat will be obtained in maternal plasma and umbilical cord plasma (when possible) at single time points, with no formal PK parameter analysis planned. PD assessments will include bile acids alongside other biomarkers.

In Part 1, ongoing monitoring of open-label safety data will be conducted by the sponsor at regular intervals per the medical and data monitoring plans as well as the routine safety surveillance activities, including but not limited to signal detection monitoring. Additionally, a chartered, independent data monitoring committee (IDMC) will review unblinded maternal and fetal safety/plasma drug level data on an ongoing basis during this study (Part 2 only), with the initial IDMC meeting (post-study start) to be held after 12 participants in Part 2 complete the study; cadence for subsequent meetings will be outlined in the IDMC charter. The sponsor plans to continue formal safety and efficacy monitoring via a chartered IDMC in Part 2; this will include AE monitoring specifications.

4.2. End of Study Definition

The end of the study is defined as the date of the last visit of the last participant in the study. The end of study for individual participants is the completion of the follow-up period after the day of delivery, including completion of follow-up Visit 2 (F2).

4.3. Scientific Rationale for Study Design

4.3.1. Overall Study Design

This is a randomized controlled study. In Part 1, open-label dosing in both cohorts enables access to real-time unblinded safety/tolerability and PK data in participants who receive the 20-mg or 80-mg doses of volixibat. For Part 2, the double-blind design is adopted to minimize systematic bias. Randomization eliminates bias and helps increase the homogeneity of participants across treatment groups.

4.3.2. Rationale for Endpoints

4.3.2.1. Efficacy Endpoints

Mean Change from Baseline to Week 3 in Total sBA

ICP is characterized by raised sBA levels. According to Williamson and colleagues (2014), the most sensitive and specific marker for diagnosis of ICP is sBA level, in particular peak sBA levels that are strongly correlated with the risk of stillbirth and other adverse perinatal outcomes (Geenes et al. 2014; Ovadia et al. 2019). Bile acids, the end products of hepatic cholesterol

metabolism, are inherently cytotoxic. In ICP, the transport of bile salts from the liver to the gallbladder is disrupted, resulting in the transport of bile salts from the hepatocytes into the blood.

The fetal complications in ICP are thought to be related to high levels of bile acids in the fetal serum. The fetus can synthesize bile acids from around 12 weeks of gestation; however, in ICP, some of the bile acids in the fetal compartment are derived from the mother. In normal pregnancy, there is a transplacental gradient for bile acids that facilitates excretion of these toxic compounds from the fetus. This gradient is reversed in ICP, leading to an accumulation of bile acids in the fetal serum and meconium. A relationship between the maternal sBA level and adverse outcomes was found, establishing that for every 1- to 2-µmol/L increase in the bile acid level, there was a 1% to 2% increase in the risk of adverse outcome (Williamson and Geenes 2014).

For participants with ICP, the reduction of sBA provides an opportunity to address both the significant burden of maternal pruritus as well as reduce the high rate of fetal morbidity and mortality. Pharmacological inhibition of IBAT has demonstrated a reduction in sBA and pruritus across multiple clinical studies involving cholestatic liver diseases (Hegade et al. 2017; Thompson et al. 2019; Bowlus et al. 2019; Gonzales et al. 2019a, 2019b; Mayo et al. 2019). Evidence from other IBAT inhibitors in cholestatic diseases supports the rationale that volixibat has the potential to lower total sBA concentrations in women with ICP and impact pruritus and other comorbidities of ICP. In clinical studies, volixibat demonstrated a consistent ability to reduce systemic bile acids, as evidenced by rapid and significant fecal excretion and consistent changes in markers, indicative of an interruption in the enterohepatic recirculation of bile acids. In Phase 1 studies, in instances where participants demonstrated elevated baseline levels of sBA, rapid and significant decreases were observed on volixibat treatment, consistent with findings from a nonclinical model of mild cholestasis.

Peak sBA levels are associated with risk of adverse perinatal outcomes; for this reason, nonfasted (i.e., postprandial) sBA levels are clinically useful for monitoring ICP because they typically reflect higher levels compared with fasting values (Geenes et al. 2014; Oruc et al. 2014). Collection of samples 30–90 minutes postprandially would be expected to capture peak serum levels in most patients (Lundasen et al. 2006).

This study assesses the primary efficacy endpoint at 3 weeks because it is clinically relevant as a timeframe within which demonstrated efficacy of a drug for ICP will be important for clinical decision making. In a large population-based, case-control study (Geenes et al. 2014), 669 patients with a singleton pregnancy were identified within the UK Obstetric Surveillance System (UKOSS) as having severe ICP; the mean gestational age was 33 weeks 4 days at time of diagnosis in these patients. Additionally, in a recent double-blind, multicenter, randomized placebo-controlled study that enrolled 605 women with ICP in the United Kingdom (Chappell et al. 2019), the median gestational age was 34.4 weeks at randomization. With the mean/median gestational ages at diagnosis occurring between 33 and 34 weeks—and with Royal College of Obstetricians and Gynaecologists (RCOG) guidelines (2011) recommending consideration of elective delivery for patients with ICP after 37 weeks based on sBA-related risks of stillbirth—approximately 3 weeks of study drug treatment corresponds to a timeframe within which demonstration of sBA reduction is expected to be impactful for clinical decision making. Furthermore, based on clinical experience with another IBAT inhibitor (maralixibat),

continued improvements in sBA are likely to be seen with relatively longer treatment durations, arguing against analysis of sBA prior to the 3-week timeframe as a primary efficacy endpoint. In Study LUM001-501, decreases in both sBA and ItchRO (observer) were observed at Week 2 and continued to decline even further at Week 4 in pediatric participants with non-truncating PFIC2, supporting the benefit of treatment durations >2 weeks for reduction of sBA and pruritus. However, given the mean/median gestational ages for ICP diagnosis (33–34 weeks), along with the requirement for randomization no later than 36 weeks 0 days in Part 2, participants are relatively more likely to be at the upper range of gestational age eligibility in this study, and as such, relatively few participants in this study will be expected to remain on treatment beyond 3 weeks.

Therefore, in addition to 3 weeks being a relevant timeframe for clinical decision making, assessment of the primary endpoint at 3 weeks of treatment is also expected to increase the likelihood of seeing a relatively larger treatment effect compared with assessment at 2 weeks, while keeping the number of participants with missing data to a reasonable minimum. Additionally, greater, statistically significant decreases in sBA and pruritus were seen in treated participants versus placebo at 2 weeks in a study of another IBAT inhibitor for a cholestatic indication (Hegade et al. 2017), supporting the likelihood of identifying a statistically significant treatment effect by 3 weeks in this study.

Mean Change from Baseline to Week 3 in Adult ItchRO

ICP is characterized by an intense, frequently intractable pruritus with typical onset in the second or third trimester of pregnancy, which negatively affects quality of life. The associated pruritus may worsen at night, resulting in insomnia and additional fatigue; the maternal symptoms may also lead to iatrogenic preterm delivery with associated fetal morbidity/mortality.

The Adult ItchRO consists of a daily eDiary; each eDiary is a single-item measure using an 11-point numerical rating scale (NRS) from 0 (no itch) to 10 (worst possible itch), where higher scores indicate greater itch intensity. The Adult ItchRO was originally developed and tested through concept elicitation and cognitive interviews with patients with PBC, a cholestatic disease with similarities to ICP in terms of clinical presentation of cholestatic pruritus (Boonstra et al. 2012). Results of the cognitive debriefing demonstrated that patients understood the items, response options, and recall period used in the Adult ItchRO Worst Itch Numeric Rating Scale (WI-NRS). The vast majority of patients interpreted the Adult ItchRO eDiary items as intended and all patients interpreted the response scale in the Adult ItchRO as intended.

This initial study confirmed the content validity of the Adult ItchRO WI-NRS in patients with PBC, and literature has shown that pruritus is a relevant and defining symptom of ICP (Bacq et al. 1997; Bergman et al. 2013). In addition, Adult ItchRO WI-NRS scales have been developed and used to support secondary endpoints for assessment of cholestatic itch (Hegade et al. 2017; Martin et al. 2019).

Prior sponsors have used the Adult ItchRO as a secondary endpoint in previous studies of cholestatic itch in participants with PSC (Study LUM001-401; CAMEO study) and PBC (Study LUM001-201; CLARITY study); these data were used to evaluate the psychometric performance of the Adult ItchRO in participants with PBC and PSC. Based on this assessment, the Adult ItchRO was found to be a reliable and valid measure that can detect small changes in pruritus, and the changes detected are clinically meaningful in patients with cholestatic liver

diseases; therefore, the Adult ItchRO is anticipated to be a suitable endpoint for clinical studies investigating potential treatments for pruritus associated with ICP.

The psychometric properties of the Adult ItchRO WI-NRS will be further evaluated in the ICP population as part of this study, as well as within a separately planned qualitative cognitive debriefing interview study including 15 additional patients with ICP. This qualitative interview study will serve to further confirm content validity in the ICP population and will also collect patient feedback regarding meaningfulness of change in Adult ItchRO WI-NRS. For further details of this instrument, refer to Section 8.2.3 and Appendix 4.

In accordance with the rationale for timing of the primary efficacy endpoint, the sponsor will assess the key secondary efficacy endpoint at 3 weeks of treatment because it is clinically relevant for clinical decision making and data from other IBAT inhibitors support the likelihood of identifying a statistically significant treatment effect by 3 weeks in this study (Hegade et al. 2017). Additionally, assessment at this time point is also expected to increase the likelihood of seeing a relatively larger treatment effect compared with assessment at 2 weeks, while keeping the number of participants with missing data to a reasonable minimum.

Proportion of Participants with a Composite Adverse Perinatal Outcome

The most devastating consequences of ICP are the associated adverse perinatal outcomes that may be life-threatening or even fatal to the fetus/infant in some cases. These include an increased risk of preterm delivery (spontaneous and iatrogenic) as well as NICU admissions for fetal/neonatal asphyxia events, respiratory distress, meconium-stained amniotic fluid, and/or other conditions in which the infant's status is severely compromised at or after birth. In some cases, intrauterine fetal demise (stillbirth) occurs before delivery.

In a recent aggregate meta-analysis, including 23 studies reporting on 5557 ICP pregnancies and 165,123 control pregnancies (Ovadia et al. 2019), ICP was associated with a higher risk of spontaneous preterm birth (odds ratio [OR]: 3.47 [95% CI: 3.06, 3.95]) and iatrogenic preterm birth (OR: 3.65 [95% CI: 1.94, 6.85]), meconium-stained amniotic fluid (OR: 2.60 [95% CI: 1.62, 4.16]), and admission to the neonatal unit or NICU (OR: 2.12 [95% CI: 1.48, 3.03]) compared with healthy pregnancies. There is extensive clinical evidence that the risks of adverse perinatal outcomes are correlated with elevated maternal sBA levels, specifically in cases where sBA levels are ≥40 µmol/L (Sepulveda et al. 1991; Williamson et al. 2001; Glantz et al. 2004; Geenes et al. 2014; Mohan et al. 2019; Ovadia et al. 2019).

Multiple outcomes may contribute significantly to the morbidity and/or mortality of the fetus and are correlated with elevated maternal sBA. Therefore, a composite perinatal outcomes measurement will be used and is designed to encompass the most impactful adverse perinatal outcomes associated with ICP in a way that is both clinically meaningful and optimizes statistical power. Because the expected rate of occurrence of any of the single event categories is expected to be too low to effectively power this study without an overwhelmingly large sample size, use of a composite endpoint can provide a substantially higher overall event rate, which allows adequate power with a more reasonable sample size. In such situations, it is common to combine several events (e.g., a composite outcome of cardiovascular death, heart attack, and stroke, an approach frequently used in studies of cardiovascular drugs) in a "composite event endpoint" where the occurrence of any of the events would constitute an "endpoint event" (FDA 2017). This concept can be generally helpful in clinical development overall, and it is even more

important when considering a clinical study involving pregnant participants to ascertain benefit while exposing as few participants to risk as possible.

The composite endpoint is designed to capture the spectrum of primary adverse perinatal outcomes associated with ICP. For further details of the specific components of this assessment, refer to Section 8.2.2.

4.3.2.2. Safety Endpoints

The safety assessments employed in this study, which include the incidence of TEAEs, SAEs, AESIs, ECIs, and AEs that lead to discontinuation, are widely used as measures to determine the overall safety profile and tolerability of a study drug.

4.3.3. Rationale for the Use of Comparator/Placebo

Placebo is used as the control to establish the frequency or magnitude of changes in clinical endpoints that could occur in the absence of active treatment.

4.4. Justification for Dose

The volixibat doses selected for this study have been determined based upon prior clinical experience with a range of oral doses between 0.5 mg QD and 80 mg BID, tested in multiple studies with healthy volunteers, obese/overweight participants, and participants with type 2 diabetes mellitus and/or NASH for up to 48 weeks. IBAT inhibitors have previously been demonstrated across multiple studies of cholestatic liver diseases to reduce sBA and pruritus in treated participants (Hegade et al. 2017; Mayo et al. 2019). IBAT inhibition in the terminal ileum results in increased fBA excretion and decreased circulating sBA. This results in a compensatory increase in hepatic bile acid synthesis, as reflected by increased levels of circulating 7αC4 (a bile acid precursor; Al-Dury and Marschall 2018). Fasting 7αC4 concentrations are a sensitive indicator of overall flux through the bile acid synthetic pathway and can be used to assess the degree of IBAT inhibition (Nunez et al. 2016).

Based on results of a volixibat Phase 1 study (VLX-103) in healthy volunteers, although some interindividual variability was noted, a dose-dependent relationship in mean fasting 7αC4 levels was seen across the full range of doses studied (5–80 mg BID). At Day 7 of volixibat dosing, higher doses of volixibat resulted in higher fasting 7αC4 concentrations: the mean 7αC4 concentration in the 80-mg BID cohort was 63% greater than in the 5-mg BID cohort, 44% greater than in the 20-mg QD cohort, 27% greater than in the 20-mg BID cohort, and 14% greater than in the 40-mg BID cohort. Furthermore, at Day 17 of volixibat dosing, the 80-mg BID dose continued to demonstrate the highest fasting 7αC4 concentrations among all doses tested. The dose dependency of 7αC4 suggests that higher doses are likely to result in greater reductions in sBA and pruritus due to dose-dependent effects on IBAT inhibition and interruption of enterohepatic circulation. Based on the sponsor's prior experience with IBAT inhibition in the Alagille syndrome clinical development program, higher doses of maralixibat indeed resulted in greater clinical reductions in sBA and pruritus, providing additional support for anticipated improved efficacy with higher doses of volixibat (Gonzales et al. 2019a, 2019b; Thompson et al. 2019).

IBAT inhibition-related GI AEs are secondary to increased fBA excretion, resulting in increased free bile acid concentrations in the lower colon. In patients with cholestatic liver disease, bile flow is reduced, and observations from ongoing programs with another IBAT inhibitor (maralixibat) in Alagille syndrome and PFIC have shown that the GI effects are generally mild and transient in nature in the setting of cholestasis. It is anticipated that when used in ICP, volixibat will have a safety and tolerability profile similar to that of maralixibat for other cholestatic liver diseases (i.e., mild/transient GI effects). Furthermore, since constipation is a very common symptom during pregnancy, it is anticipated that diarrhea associated with IBAT inhibition will likely be less frequent and less severe in patients with ICP compared with previously tested populations.

No dose-dependent safety concerns were noted across the healthy volunteers in Study VLX-103; however, compared with lower doses, moderate volixibat-related cramping was noted more frequently at the 40-mg and 80-mg doses BID, although not in a dose-dependent fashion. Notably, no diarrhea or cramping AEs led to any study drug discontinuations in Study VLX-103, including at the 80-mg BID dose. In conjunction with the dose-dependent 7αC4 increases, which suggest optimum IBAT inhibition at 80 mg BID compared with lower doses, these data support that 80 mg BID is an acceptable dose to be tested in Part 1 of Study VLX-401.

At Day 7 of volixibat dosing in Study VLX-103, fBA excretion at the 20-mg BID dose was superior compared with fBA excretion at the 2 lower doses (5 mg BID and 20 mg QD); median fold increases in fBA excretion from baseline were 5.5 (5 mg BID), 2.1 (20 mg QD), 11.7 (20 mg BID), 11.2 (40 mg BID), and 8.3 (80 mg BID). In combination with the 7αC4 data, these data suggest that dosing below 20 mg BID is likely to result in a markedly inferior pharmacologic effect on reductions in sBA and pruritus while offering no benefit in terms of safety or tolerability because no marked differences in frequency/severity of (or discontinuations due to) GI AEs were noted in Study VLX-103 in the 5-mg BID or 20-mg QD cohorts compared with the 20-mg BID cohort. Therefore, 20 mg BID is an appropriate lower boundary for initial doses to be tested in Part 1 of Study VLX-401.

With regard to BID versus QD dosing, a prior volixibat study (SHP626-101) demonstrated improved fBA excretion with BID dosing compared with QD dosing for an equivalent daily exposure (i.e., mean fBA excretion was greater for 5 mg BID compared with 10 mg QD). BID dosing provides greater coverage of IBAT over a 24-hour period, supporting the likelihood of relatively increased efficacy with a BID dosing schedule. Based on modeling, predicted GI lumen concentrations of volixibat are expected to provide full IBAT inhibition for ~12 hours following a 20-mg dose; thus, 20 mg BID would be expected to provide 24-hour coverage of the IBAT transporter on average. BID dosing has also been shown to result in greater treatment effects with another IBAT inhibitor, maralixibat, where improvements in sBA and pruritus were observed when the dose regimen was doubled from QD to BID in pediatric cholestasis.

Based on available safety, tolerability, and PD data, and further supported by data demonstrating only sporadically detectable volixibat concentrations (with most below the LLOQ) in human plasma at the doses proposed, 20-mg BID and 80-mg BID are likely to represent an appropriate range of lower and higher dosages to study in Part 1 for treatment effects while being well tolerated and maintaining an acceptable safety profile in ICP for participants and fetuses. Although 20 mg BID and 80 mg BID are the initial dosages, a dose reduction of up to 75% of the original assigned dose, as outlined in Section 6.2, may also provide the opportunity for de facto

exploration of 3 additional dosages (i.e., 5 mg BID, 10 mg BID, and 40 mg BID) in Part 1, should dose reductions be warranted for safety or tolerability considerations in some participants. Based upon thorough review of dose/exposure/response relationships for all doses explored in Part 1 at the interim analysis, an appropriate dose for Part 2 will be selected.

5. STUDY POPULATION

Participants must meet all inclusion criteria and no exclusion criteria at screening and at baseline (randomization). Laboratory results that are qualifying at screening do not need to be repeated at the baseline visit unless specified in the SoA (Table 1).

5.1. Inclusion Criteria

Participants who meet all of the following criteria will be eligible for the study.

Informed Consent

1. Provide signed informed consent as described in Section 11.3 and be willing to comply with all study visits and requirements through end of study, including the follow-up period (F2)

Age

2. Female aged ≥18 and ≤45 years with viable pregnancy of 20 weeks 0 days or above.

   Part 1: Singleton gestation and no more than 37 weeks 0 days (inclusive) or twin gestation and no more than 35 weeks 0 days (inclusive) at the baseline visit (Day 0)

   Part 2: Singleton gestation and no more than 36 weeks 0 days (inclusive) at the baseline visit (Day 0)

   Note: Confirmation of eligibility is based upon estimated gestational age required per National Institute for Health and Care Excellence (NICE) guidelines before randomization: crown-rump length (CRL) from early ultrasound or head circumference on ultrasound if CRL >84 mm.

Type of Participant and Disease Characteristics

3. Diagnosis of ICP, as characterized by the following:

   Onset of pruritus during pregnancy with no known etiology other than ICP (e.g., pruritic urticarial papules and plaques of pregnancy, pruritic folliculitis, uncontrolled thyroid disease, etc.)

   sBA level ≥10 µmol/L at any point during the current pregnancy

   Absence of known pathology that may produce similar laboratory findings/symptoms; these include but are not limited to other cholestatic disorders such as PBC and PSC

   Note: Suspicion of transient hypercholanemia should be discussed with medical monitor to confirm participants eligibility.

4. Part 1:

   sBA of ≥10 µmol/L as assessed by the central laboratory during the screening period or local laboratory at any time during the current pregnancy

   Part 2:

   sBA level ≥20 µmol/L as assessed by the central laboratory during the screening period

   Documentation of historic or local sBA level is required to confirm participant eligibility before randomization.

   Laboratory assessments may be repeated at the investigator's discretion before randomization.

   Participants already on UDCA at the time of the screening visit shall be eligible for Part 1.

5. Willing and able to refrain from the use of any of the following for the duration of the study: statins, rifampin, bile acid sequestrants (e.g., cholestyramine), peroxisome proliferator-activated receptor (PPAR) agonists/fibrate drugs (e.g., fenofibrate, bezafibrate), S-adenosyl methionine

   Participants requiring local/systemic corticosteroids or selective serotonin reuptake inhibitors (e.g., sertraline) for the management of non-cholestatic, non-pruritic conditions must have been on a stable dose for at least 1 week before screening and be willing to remain on a stable dose for the duration of the study to be considered eligible.

   Administration of isolated doses of corticosteroids for obstetric indications (e.g., promotion of fetal lung maturity for anticipated preterm delivery) is permitted

6. Part 2 only: Willing and able to enter daily patient-reported outcome (PRO) information in an eDiary for the duration of the study and completes ≥70% of the eDiary ItchRO assessments during the screening period. A minimum of 4 once-daily ItchRO entries are required during screening.

7. Part 2 only: Qualified pruritus reflected by an average daily Adult ItchRO score ≥4 overall during the screening period

5.2. Exclusion Criteria

Any one of the following will exclude potential participants from eligibility for the study:

Medical Conditions

1. At the time of either the screening or baseline visit, decision has already been made to deliver within the next 7 days, for any indication

2. Presence of triplets or higher multiple gestation, known placenta accreta, complete placenta previa, premature rupture of membranes (PROM) at any gestational age prior to randomization, cervical incompetence, history of prior spontaneous birth at ≤34 weeks not secondary to known or suspected ICP, or other condition (in the

opinion of the investigator/medical monitor [refers to sponsor or contract research organization (CRO) medical monitor throughout]) likely to result in spontaneous or iatrogenic delivery before 37 weeks

3. Known non-reassuring fetal status based upon antepartum testing (e.g., NST/CTG or BPP) at or within 7 days before the baseline visit
4. Known fetal anomaly likely to result in intrauterine fetal demise or neonatal death within the first 30 days of life
5. History or evidence, with the exception of ICP, of current underlying cholestatic liver disease at screening or baseline, including but not limited to:
    - PSC or secondary sclerosing cholangitis (SSC)
    - Primary biliary cholangitis (PBC)
    - Immunoglobulin G4-related cholangitis
    - Ascending cholangitis
    - Clinical evidence or suspicion of dominant strictures that are considered clinically relevant in the opinion of the investigator/medical monitor or current or planned placement of percutaneous drain/biliary stent at screening
6. Evidence of other hepatobiliary conditions at screening or baseline, including but not limited to:
    - Active hepatitis A infection
    - Active hepatitis B infection as defined by the presence of hepatitis B surface antigen (HBsAg) or presence of hepatitis B virus DNA
    - Hepatitis C as defined by the presence of hepatitis C virus (HCV) antibody and positive HCV RNA. Documented cured HCV infection is acceptable if >2 years earlier than the time of randomization
    - History or evidence of autoimmune hepatitis, Wilson disease, alpha-1-antitrypsin deficiency, hemochromatosis, or drug-induced liver disease (as defined on the basis of typical exposure and history). A history of temporary, reversible cholestasis associated with medication use (e.g., hormones, antibiotics, etc.) is not exclusionary.
    - Evidence or clinical suspicion of cirrhosis, including decompensated cirrhosis (e.g., hepatic encephalopathy, portal hypertension, hepato-renal syndrome, hepato-pulmonary syndrome, esophageal varices, ascites)
    - Suspected or proven cholangiocarcinoma or hepatocellular carcinoma
    - History of liver transplantation
    - Confirmed or suspected NASH as determined by the investigator
    - Acute fatty liver of pregnancy

- Severe preeclampsia/hemolysis, elevated liver enzymes, and a low platelet count (HELLP) syndrome; suspected hemolytic anemia

  Note: The presence of mild preeclampsia, gestational hypertension, or chronic hypertension is not considered exclusionary for study participation if the condition is adequately managed and there is no known plan for delivery within the next 7 days.

- Alcohol-related liver disease
- Known diagnosis of Gilbert syndrome

  Note: The presence of gallstones or a prior history of cholecystectomy is NOT considered exclusionary.

7. History or presence of known or suspected inflammatory bowel disease (e.g., Crohn's, ulcerative colitis)
8. History of small bowel resection or bariatric surgery (e.g., gastric bypass, banding, or sleeve), or surgery resulting in disruption of the enterohepatic circulation
9. Part 2 only: Presence of any concurrent underlying condition with unstable pruritus (e.g., unstable atopic dermatitis, psoriasis, urticaria, psychogenic pruritus, etc.)

   Note: Participants with stable pruritic conditions (as above) that predate pregnancy and who have been on stable doses of treatment medications for the pruritic condition for ≥4 weeks before screening may be considered eligible
10. Anticipated need for liver transplantation within 12 months (365 days) after randomization
11. Active infection that, in the opinion of the investigator or medical monitor would preclude participation in the study for participant safety reasons

    Note: An active COVID-19 infection is considered exclusionary unless approved by the medical monitor.
12. Unstable and/or serious medical disease that is likely to impair the participant's ability to participate in all aspects of the study or result in substantially shortened life expectancy (e.g., malignancy, end-stage heart failure).
13. Known diagnosis of HIV infection or HIV antibody positivity
14. Clinically relevant alcohol use disorder or substance abuse within 12 months (365 days) before screening. Alcohol use disorder for this study is defined as ≥2 standard drinks on average per day for women, with a standard drink defined as 1.5 oz (1 shot) of liquor, 5 oz of nonfortified wine, or 12 oz of beer (as defined by the National Institute of Alcohol Abuse and Alcoholism; 1 oz=29.57 mL).

Diagnostic Assessments

15. Participant has the following laboratory parameters at the screening visit, as determined by the central laboratory:

    - Platelet count ≤100,000/mm^3

- Serum creatinine ≥77 µmol/L (0.87 mg/dL)
- AST or ALT ≥5 × ULN
- Total bilirubin >1.3 mg/dL
- INR >1.3 unless due to therapeutic anticoagulation

16. Evidence or preexisting history of atrial fibrillation, atrial flutter, complete bundle branch or heart block, Wolff-Parkinson-White syndrome, or other abnormality that is deemed clinically significant and/or likely to put participants at unacceptable risk to study participation, as determined by the investigator or the medical monitor.
17. Except as defined elsewhere in the inclusion or exclusion criteria, abnormal laboratory results that are deemed to be clinically significant and put participants at unacceptable risk for study participation, as determined by the investigator or the medical monitor.

NOTE: Investigators have the discretion to repeat assessments if they believe there is a reasonable possibility that the results were spurious or otherwise confounded. Repeat assessments (no more than 1 per laboratory parameter, under appropriate conditions) must be conducted within the screening period.

Prior/Concomitant Therapy

18. Use within 5 half-lives before randomization of any of the following medications:
    - Methotrexate
    - Sodium phenylbutyrate
    - PPAR agonists (e.g., fibrates)
    - Any investigational drug
    - Known hepatotoxins (e.g., cyclosporine, tacrolimus, Janus kinase [JAK] inhibitors [when dosed systemically], pancreatic lipase inhibitors [when dosed systemically], drugs [including any medicinal products, herbs, etc.] that are known to cause drug-induced liver injury [DILI] at the therapeutic labeled doses)
19. Use within 5 half-lives before randomization of any of the following medications:
    - UDCA (Part 2 only; UDCA use is permitted in Part 1)
    - Rifampicin
    - Bile acid/lipid-binding resins (e.g., cholestyramine)
    - S-adenosylmethionine
    - Any other medications for the treatment of cholestasis – on- or off-label use
20. Use within 5 half-lives before randomization of any of the following (when dosed systemically):
    - Progesterone supplementation

- Cholestasis-inducing medications, including but not limited to erythromycin, amoxicillin-clavulanate, angiotensin-converting enzyme (ACE) inhibitors, terbinafine (Larson 2020), or prochlorperazine (Prochlorperazine USPI)

21. Inability to tolerate antidiarrheal medications
22. Previous use of an IBAT inhibitor

Other Exclusions

23. Breastfeeding at the time of the screening visit or planning to breastfeed at any time starting with the screening visit through end of the treatment period
24. Known intolerance/hypersensitivity to volixibat or its components
25. Confirmed positive urine drug/alcohol screen at any time during pregnancy, including at the screening visit
26. Participating in another ongoing interventional clinical study at screening or planning to participate in another contemporaneous interventional clinical study while participating in this study; contemporaneous participation in studies that do not involve pharmaceuticals, devices, or other interventions and do not interfere with this study's (OHANA) SoA (i.e., observational studies, registries) are permitted
27. History of nonadherence to medical regimens, unreliability, medical condition, mental instability, or cognitive impairment that, in the opinion of the investigator or medical monitor, may interfere with the interpretation of study results, could compromise the validity of informed consent, compromise the safety of the participant, or lead to nonadherence with the study protocol or inability to conduct the study procedures.
28. Participant is in a dependent relationship with or has an immediate family member who is a study site employee that is involved in the conduct of this study (e.g., spouse, parent, child, or sibling) or participant is a study site employee.

5.3. Lifestyle Considerations

There are no diet or activity restrictions for this study. Alcohol use is strongly discouraged because there is no known amount of alcohol that can be safely consumed during pregnancy (CDC 2020).

5.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study drug. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

5.5. Rescreening Considerations

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened in the following situations:

- Participant wishes to be rescreened upon reaching 18 years of age (Inclusion Criterion 2)
- Participant's first screening sBA level did not meet Inclusion Criterion 3 or 4
- Participant had an active infection at screening but wants to rescreen after it has resolved (Exclusion Criterion 11)
- Participant recently discontinued prohibited medication and wants to return after the relevant exclusionary timeframe has elapsed for rescreening (Exclusion Criterion 18, 19, or 20)
- Participant screen failed for other reasons, but the sponsor or designee approved rescreening
- Part 2 only: Participant failed to enter at least 70% of eDiary ItchRO assessments **and** at least 4 ItchRO scores during screening but wants to try again (Inclusion Criterion 6)
- Part 2 only: Participant had been on stable antipruritic medication(s) for a non-ICP pruritic condition for <4 weeks at first screening but wants to rescreen when it has been ≥4 weeks (Exclusion Criterion 9)

Note: For Part 2, participants who screen fail solely on the basis of Inclusion Criterion 7 (i.e., do not have average Adult ItchRO ≥4 overall during the screening period) will not be permitted to rescreen.

If rescreening occurs within 3 weeks of previous screening, investigators have the option of repeating only hematology and chemistry, coagulation panel, fetal ultrasound, and perinatal assessment (repeat of urinalysis is optional). For sBA, refer to Inclusion Criteria 3 and 4.

Repeat of the following tests from the central laboratory for this study may be waived at rescreening (provided the results were within normal limits): HIV, HBsAg, HCV antibody, TSH, drug and alcohol screening, vitamins A, D, and E, lipid panel, and/or glycated albumin.

5.6. Replacement Strategy

A participant who discontinues from study drug or withdraws from the study will not be replaced.

6. TREATMENTS

6.1. Investigational Product

Volixibat in capsules at doses of 20 mg and 80 mg and matched placebo will be supplied (in a blinded fashion for Part 2) for oral dosing BID. As much as possible, the morning dose (4 capsules) will be administered ~30 minutes before breakfast and the evening dose (4 capsules)

will be administered 30 minutes before dinner to cover the luminal bile acid release associated with meals.

The sponsor or designee will provide the investigator and site with additional investigational product information, including dosage form, packaging, storage conditions, and labeling in the Pharmacy Manual. Volixibat may be provided as multiple capsules per dose. The packaged study drug will be labeled in accordance with country and regional specific regulatory requirements, and supplies will be shipped in accordance with the Pharmacy Manual.

6.2. Dose Modification

If a participant experiences an SAE believed to be related to study drug or a Grade 3 AE believed to be related to study drug, temporary interruption of dosing should be considered; see Section 7.1 for additional information on situations requiring permanent discontinuation of study drug. When possible, the medical monitor should also be notified within 24 hours after any treatment-related AE that is Grade ≥3. Dose interruptions for Grade 1 or Grade 2 AEs, including diarrhea, may be permitted in limited situations if the investigator deems it medically appropriate (e.g., prolonged symptoms with no response to adjunctive treatment, intolerance of AEs to the point that participant is considering early termination from study, etc.). In general, rechallenge (i.e., resumption of dosing) may be considered for an AE if an alternative cause for the event is discovered or the event has resolved or returned to baseline severity; any concerns regarding rechallenge should be discussed with the medical monitor. If a participant experiences an SAE or a Grade 3 AE believed not to be related to study drug, temporary interruption of study drug or dose reduction may still be considered with medical monitor or sponsor designee approval.

A dose reduction of up to 75% of the original assigned dose is permitted in a stepwise reduction. For example, if any one of the following criteria are met, a dose reduction of up to 50% is allowed and then another reduction to 25% of original dose level may occur:

- ≥1 episode of treatment-related cramping/abdominal pain requiring an unscheduled visit for threatened preterm labor
- Any treatment-related GI AE resulting in secondary dehydration and/or hospitalization
- For any participant who complains of intolerability to study drug that results in consideration of withdrawal from study drug, a dose interruption or reduction should be offered prior to early discontinuation from treatment.
- Dose reductions for events not meeting the above criteria are generally not recommended but may be permitted in limited situations with approval from the medical monitor.

For Part 2, all dose modifications and rechallenges will be performed in a blinded manner.

Upon satisfactory resolution of the event(s) driving the dose modification as deemed by the investigator, rechallenge with the original dose level may be considered. All rechallenges must be approved by the medical monitor.

For any treatment-related GI AEs, such as diarrhea, investigators are strongly encouraged to initiate therapy, such as loperamide, before a dose reduction is considered for tolerability reasons. Additional information on the management of diarrhea is provided in Section 6.8.1.1.

All participants who experience related Grade ≥3 AEs shall be followed up via telephone and/or clinic visits (unscheduled if necessary) every 2–4 days until resolution. If the participant's clinical status worsens, study drug shall be discontinued.

Additional information on the management of abnormal liver function tests, including criteria for potential dose interruption, is provided in Appendix 3.

6.3. Method of Treatment Assignment and Management

6.3.1. Randomization Procedures

Individual participant treatment is automatically assigned by an interactive response technology (IRT) system.

- For Part 1, participants will be randomized with stratification (based on background use of UDCA or not, gestational age [<32 weeks or ≥32 weeks] at randomization, and presence/absence of gestational diabetes) after confirmation of study eligibility in a 1:1 ratio via a computer-generated randomization schedule to receive volixibat 20 mg BID or 80 mg BID.
- For Part 2, a dynamic allocation method, introduced by Pocock and Simon (1975) will be adopted to balance participant assignment between treatment arms based on the following factors: gestational age (<32 weeks or ≥32 weeks) at randomization, presence/absence of gestational diabetes, and highest sBA before randomization (<40 µmol/L, ≥40 µmol/L to <100 µmol/L, or ≥100 µmol/L) after confirmation of study eligibility in a 1:1 ratio via a computer-generated randomization schedule to receive the selected volixibat dose (determined at the interim analysis) or placebo BID

The participant's randomization number represents a unique number corresponding to the treatment allocated to the participant.

6.3.2. Interactive Response Technology for Study Drug Management

An IRT system will be used for screening and enrolling participants, randomization, study drug supply dispensation and management, inventory management and supply ordering, study drug expiration tracking and management, and emergency unblinding. Please refer to the study manual for additional details regarding the IRT.

The investigator or designee will access the IRT at the screening visit (Visit 0) to record participant-specific information (i.e., unique participant number, age, etc.). At the baseline visit (Visit 1), the investigator or designee will again access the IRT to either document a screen failure, or if the participant has met all entry criteria, to randomize the participant. For randomized participants, the IRT will provide 1 or more bottle identification number(s) to dispense for treatment.

A user manual with specific functions and instructions for the IRT will be provided to the site, and site personnel will be trained on its use.

6.4. Blinding

Placebo capsules will contain compendial excipients that are commonly used in solid oral dosage formulations without the active API. The placebo capsules visually match the active drug product. To maintain the blind, all packaged study drug components will be identical.

For Part 2, investigators will remain blinded to each participant's assigned study drug throughout the course of the study.

6.5. Unblinding Study Drug Assignment

For Part 2, the treatment assignment must not be unblinded during the study except for expedited safety reporting and in emergency situations where the identification of the study drug is required for further treatment of the participant. The investigator should make an effort to contact the medical monitor before emergency unblinding occurs or as soon as possible after the investigator has been unblinded without revealing the treatment assignment to the sponsor or designee. In any event, the sponsor or designee must be informed about the code break as soon as possible.

If the treatment assignment is unblinded, the date and the signature of the person who was unblinded and the reason for unblinding will be recorded in the source documents. Upon breaking the blind in emergency situations, the participant will be withdrawn from study drug but should continue to be followed in the study for safety purposes as outlined in Section 7.1. Unblinding will be processed through the IRT vendor, and instructions will be available in the user manual.

6.6. Study Drug Accountability

Study drug will be dispensed at the study visits summarized in the SoA (Table 1). Returned study drug will not be re-dispensed to participants.

Investigators will be provided with sufficient amounts of the study drug to carry out this protocol for the agreed number of participants. The investigator or designee will acknowledge receipt of the study drug, documenting shipment content and condition. Accurate records of all study drug dispensed, used, returned, and/or destroyed must be maintained.

The investigator has overall responsibility for dispensing study drug. Where permissible, tasks may be delegated to a qualified designee (e.g., a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. Study drug will be dispensed at baseline and at each visit in quantities sufficient for dosing at least until the next scheduled visit. If necessary, direct-from-site to participant shipment of study drug is allowed between visits.

The investigator or designee will dispense the study drug only to participants included in this study and following the procedures set out in the study protocol. Each participant will be given only the assigned study drug. All dispensed study drug will be documented in the IRT system.

The investigator is responsible for ensuring the retrieval of all study supplies from participants. Participants must be instructed to bring their unused and empty or used study drug packaging to every visit. Drug accountability must be assessed at the capsule level. The pharmacist or designee will record details on the drug accountability form.

Other than study drug dispensed to participants, no stock or returned inventory may be removed from the site where originally shipped without prior knowledge and written consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The sponsor or its representatives must be permitted access to review the supply storage, distribution procedures, and records provided that the blind of the study is not compromised by such access.

At the end of the study or as instructed by the sponsor, all unused stock, participant-returned study drug, and empty or used study drug packaging are to be returned or destroyed. Study drug must be counted and verified by clinical site personnel and the sponsor (or designated clinical research organization) before return or destruction. Shipment return or destruction forms must be completed before shipment from the site or destruction. Shipment of all returned study drug must comply with local, state, and national laws.

On the basis of entries in the site drug accountability forms/IRT, it must be possible to reconcile study drug delivered with those used and returned. All study drugs must be accounted for, and all discrepancies investigated and documented to the sponsor's satisfaction.

The investigator or designee must confirm that appropriate temperature conditions have been maintained during transit for all study drug received and that any discrepancies are reported and resolved before use of the study drug.

Only participants enrolled in the study may receive study drug, and only authorized site staff may supply or administer study drug. All study drugs must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the investigator and authorized site staff.

Further guidance and information for the final disposition of unused study drug are provided in the study manual.

6.7. Participant and Treatment Compliance

Participant compliance with study procedures and treatment compliance will be assessed at each treatment visit by the site staff. If a participant misses >25% of the assigned number of capsules weekly for 2 consecutive weeks because of non-safety related issues, investigators are encouraged to retrain the participant on treatment compliance before considering discontinuation from study treatment. Any concerns with compliance should be discussed with the sponsor or designee.

6.8. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of screening or receives at any time during the study must be recorded, along with:

- Reason for use
- Dates of administration, including start and end dates
- Dosage information, including dose and frequency

Between at least 28 days before the screening visit and end of treatment, only those medications that are allowed (see Section 6.8.1) and are deemed necessary to maintain the participant's health status are permitted for concomitant use. The use of over-the-counter, dietary supplements, and herbal medications during study conduct is strongly discouraged, unless their use is considered necessary to maintain a participant's current health status.

The investigator is strongly encouraged to discuss the use of concomitant medications with the medical monitor before initiation of any medications, unless the participant requires immediate medical attention.

Volixibat has the potential to inhibit the organic anion transporter OATP2B1 in the GI tract. This could potentially lower the plasma levels of orally administered drugs that are absorbed via the OATP2B1 transporter in the gut. This should be considered before starting or continuing administration of drugs that are known substrates of this transporter. See Appendix 10 for a list of known OATP2B1 substrates.

6.8.1. Allowed Medications

6.8.1.1. Concomitant Medications for Common Volixibat Side Effects

Transient diarrhea, abdominal cramping, and/or nausea are commonly associated with IBAT inhibitors, such as volixibat. Given the importance of minimizing premature discontinuations to maintain study integrity, every effort should be made to treat these typically transient symptoms before resorting to discontinuation of study drug. Participants are strongly encouraged to take approved medications for GI symptoms (i.e., antidiarrheals, antiemetics, abdominal discomfort, etc.) as outlined in Table 2; in the event inadequate relief is obtained with allowed medications, the medical monitor should be consulted regarding potential other options that may be permitted.

An investigator or obstetrician's clinical judgment should be used to determine whether abdominal cramping or discomfort warrants prompt evaluation to rule out threatened preterm labor or other possible obstetric complications.

Table 2 Allowed Medications and Therapies for Common Volixibat Side Effects

Indication	Allowed Medications and Therapies
Diarrhea	Loperamide[a], over-the-counter soluble fiber supplements (e.g., methylcellulose, psyllium)
Nausea[b]	Pyridoxine, metoclopramide, ondansetron
Abdominal cramping/discomfort	Oral hydration

[a] Prompt use of loperamide is strongly encouraged if participant experiences gastrointestinal-related adverse events, such as diarrhea.

[b] Many other antiemetics have antihistaminic properties, which may confound pruritus assessments. In the event of inadequate relief with allowed antiemetics in this table, the medical monitor should be consulted, when possible, prior to initiating other antiemetic therapies.

6.8.1.2. Rescue Medication

Use of the following medications may be considered as "rescue" for the treatment of worsening ICP, only under limited circumstances and after discussion with the medical monitor:

- Antipruritics (antihistamines or topical antipruritics, such as menthol, pramoxine, or corticosteroids)
- UDCA – for participants who were not on UDCA at screening, or an increase in dose for participants in Part 1 (who were on a stable dose of UDCA at screening)

Whenever possible, the use of concomitant rescue medications should not be initiated for at least 3 weeks after the initiation of study drug. The following criteria may be considered in making decisions regarding initiation of rescue medication:

- For initiation or an increase in existing dose of oral antihistamines or topical antipruritics:
  - Intolerable pruritus as the sole symptom of worsening cholestasis (i.e., without jaundice)
- For initiation or an increase in existing dose of UDCA:
  - Onset of symptoms consistent with clinically significant worsening cholestasis, such as new onset of jaundice

    If worsening pruritus is the sole symptom of worsening cholestasis, a trial of oral antihistamines or topical antipruritics (initiation of, or an increase in existing dose) should be attempted before initiating UDCA.
  - Laboratory criteria: Sustained increase of sBA >100 µmol/L

The rationale for initiation of rescue medication, start/stop dates, and times of rescue medication administration as well as the name and dosage regimen of the rescue medication must all be recorded in the electronic case report form (eCRF).

6.8.2. Prohibited Medications

None of the following medications are allowed during the conduct of the study:

- Any investigational drug, or medical device
- IBATi other than volixibat
- UDCA (except as permitted in Part 1 and/or in Section 6.8.1.2) or other therapeutic bile acids
- Methotrexate
- Statins
- Bile acid/lipid-binding resins or sodium phenylbutyrate
- Rifampin
- PPAR agonists/fibrate drugs

- S-adenosylmethionine
- Any medications for the treatment of cholestasis – on- or off-label use
- Known hepatotoxins (e.g., cyclosporine, tacrolimus, JAK inhibitors [when dosed systemically], pancreatic lipase inhibitors [when dosed systemically], drugs [including any medicinal products, herbs, etc.] that are known to cause DILI at the therapeutic labeled doses)
- Progesterone supplementation
- Cholestasis-inducing medications, unless required short-term for safety reasons and with medical monitor approval (e.g., short-term use of antibiotics for group B streptococcus prevention if no viable alternatives are available)
- Initiation or change in dosing of prior antipruritic therapies (except as permitted in Section 6.8.1.2)

6.8.3. Treatment after End of Study

There is no study-specified treatment after the end of study.

7. DISCONTINUATION OF STUDY DRUG AND PARTICIPANT WITHDRAWAL CRITERIA

7.1. Discontinuation of Study Drug

Participants may elect to discontinue study drug at any time for any reason.

Whenever possible, participants who discontinue study drug should continue in the study on a weekly visit schedule until completion of F2, 28 (±3) days after delivery as outlined in the SoA (Table 1).

A participant will be discontinued from study drug for any of the following reasons:

- Withdrawal of consent by the participant
- Emergency unblinding of treatment assignment by the investigative site
- Criteria for stopping drug due to suspected DILI are met (refer to Appendix 3)
- Any AE or progression of ICP refractory to rescue medication use that leads the investigator to decide that the participant should be withdrawn from study drug, including but not limited to the following:

  Any AE of Common Terminology Criteria for Adverse Events (CTCAE) Grade 4, related to study drug
- Evidence of hepatic decompensation, including variceal bleeding, hepatic encephalopathy and/or ascites

- In the opinion of the investigator or study medical monitor, it is medically unacceptable or otherwise not in the participant's best interest to continue with study drug
- Gross noncompliance, including failure to adhere to the study requirements as stated in the study protocol.

Refer to the SoA (Table 1) for data to be collected at the time of end of treatment and follow-up and for any further evaluations that need to be completed.

7.2. Participant Withdrawal from Study

A participant may withdraw from the study at any time for any reason without prejudice to future medical care by the physician or at the institution. The sponsor or designee may discontinue the participant from the study at any time. If an investigator determines that a participant's discontinuation from study is warranted, such action should be discussed with the sponsor or designee, when possible, in advance of the discontinuation. The reason for termination and the date of stopping study drug must be recorded in source documents.

Participants who discontinue from the study for safety reasons are followed as long as clinically indicated or until a safety finding is considered to be permanent.

Participants who discontinue from the study will not be replaced.

If a participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

A participant who withdraws from the study may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

The reason for termination and the date of stopping study drug must be recorded in source documents. The evaluations listed for the early termination visit are to be performed as completely as possible for any participant who discontinues from study participation.

Data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed are outlined in the SoA (Table 1).

7.3. Lost to Follow-Up

A participant will be considered lost to follow-up if she fails to return for 3 or more scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee should make every effort to regain contact with the participant (where possible, 3 telephone

calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.

- If the participant continues to be unreachable, the participant will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the medical monitor immediately upon occurrence or awareness to determine whether the participant should continue or discontinue study drug.

Adherence to the study design requirements, including those specified in the SoA (Table 1), is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria.

Procedures conducted as part of the participant's routine clinical management (e.g., sBA) and obtained before signing of the informed consent form (ICF) may be used for screening purposes, provided the procedures meet the protocol-specified criteria and are performed within the time frame defined in the SoA (Table 1).

8.1. Administration and General Procedures

8.1.1. Informed Consent

The investigator or medically qualified designee (consistent with local requirements) will obtain informed consent, as described in Section 11.3, from each potential participant before she participates in this study.

8.1.2. Assignment of Participant Identification Number

All consented participants will be assigned a unique number that will be used to identify the participant for all screening procedures and will serve as the Participant ID number following randomization. Each participant will be assigned only 1 participant number for each screening. Participant numbers must not be re-used for different participants.

8.1.3. Inclusion/Exclusion Criteria

At the screening and baseline visits, all inclusion (Section 5.1) and exclusion criteria (Section 5.2) will be reviewed by the investigator to ensure that the participant qualifies for the study.

8.1.4. Demography

Participant demographic information including sex, age (date of birth), and race, as allowed per local regulations, will be collected during the screening visit.

8.1.5. Prior and Concomitant Treatments

Specific prior treatments and therapies administered for treatment of pruritus or ICP will be collected. All concomitant treatment and therapies for any indication will also be collected per the SoA (Table 1). All prior and concomitant treatment information must be recorded in the participant's source documents.

8.1.6. Medical, Menstrual, and Obstetric History

A medical history will be obtained by the investigator or qualified designee. All clinically or medically relevant information must be recorded, regardless of how much time has elapsed since the date of diagnosis. Menstrual history (e.g., last menstrual period [LMP] and date of positive human chorionic gonadotropin test), and obstetric history (e.g., previous pregnancies including terminations of pregnancy and/or spontaneous abortions, genetic screenings, aneuploidy screening, teratogen exposures since LMP/pregnancy, infections [i.e., Covid-19, Zika, HIV, or hepatitis], travel outside the country, history of in vitro fertilization or other fertility treatments, history of cerclage placement) will be obtained.

8.1.7. Estimated Gestational Age Assessment

Confirmation of estimated gestational age is required during the screening period (i.e., before randomization). Records of gestational age confirmation by 1st or 2nd trimester ultrasound should be reviewed; ultrasound should be performed during the screening period if not previously done or if prior records are unavailable. Acceptable criteria include CRL from early ultrasound or head circumference on ultrasound if CRL is >84 mm, supporting a gestational age between 20 weeks 0 days and the following:

- No more than 37 weeks 0 days or 35 weeks 0 days for singleton or twin gestation, respectively, inclusive, at the baseline visit (Day 0) for Part 1
- No more than 36 weeks 0 days, inclusive, at the baseline visit (Day 0) for Part 2

8.1.8. Screening Visit

The screening visit(s) will occur up to 10 days prior to randomization. The investigators may randomize participants as soon as participant's eligibility is confirmed. All screening procedures are expected to be completed within the 10-day screening period. See SoA (Table 1) for details.

8.1.9. Baseline Visit

The baseline visit should occur in the morning, whenever possible. After eligibility for randomization is confirmed, the following assessments must be performed in order; please refer to the SoA (Table 1) for details.

1. PRO assessments
2. Nonfasting (e.g., postprandial) blood draw for sBA and other laboratories, per the SoA
3. First dose of study drug under direct observation, with fetal monitoring pre- and post-dose (see Section 8.4.2 for details). The first dose of study drug must be administered only after the sBA blood draws have been completed.

Note: Participants may have the option to complete the baseline visit PRO assessments (i.e., 5D-itch scale, CSS, EQ-5D-5L, PROMIS SF v1.0-Fatigue 7a, PROMIS SF v1.0-Sleep Disturbance, PIS-Itch) at home before they arrive onsite for the baseline (Day 0) visit.

Timing of other required procedures at the baseline visit is at the investigator's discretion.

8.1.10. Randomization

Participants who are eligible for Part 1 will be randomized (1:1) via IRT to receive either volixibat 20 mg BID or volixibat 80 mg BID.

Participants who are eligible for Part 2 will be randomized (1:1) via IRT to receive the volixibat dose BID selected at the interim analysis following Part 1 or matching placebo.

8.1.11. Study Drug Administration

Participants are to take their study drug as prescribed. As much as possible, each dose should be administered ~30 minutes before meals to better cover the luminal bile acid release associated with meals.

The first dose of study drug will be administered under observation in the clinic; monitoring of fetal well-being will be performed before and after dosing via NST/CTG or BPP or auscultation/visualization of fetal cardiac activity if NST/CTG and/or BPP are not feasible due to gestational age (see Section 8.4.3 for additional details).

8.1.12. Management of Timing for Possible Elective Delivery

Recommendations for timing of delivery in ICP pregnancies are inconsistent in guidelines across the world (see Section 2.2). For the purposes of consistency within this study and unless contraindicated for safety reasons for an individual participant, whenever possible investigators are advised to follow the RCOG guidelines for the management of ICP in effect at the time of a participant's randomization when making decisions regarding timing of delivery.

Recommendations from RCOG's COVID-19 guidance for maternal medicine services (2020) include:

- If bile acids are below 100 µmol/L at diagnosis, repeat bile acid testing should be offered at any in-person appointments held from 34 weeks of gestation, or at 37 weeks of gestation as a minimum, in order to guide delivery timing. If bile acids remain below 100 µmol/L, a planned birth at 39 weeks should be considered.
- If bile acids are 100 µmol/L or above, a repeat blood test for sBAs at 34 weeks of gestation should be offered. If they remain raised, the benefits and risks of planned birth at 35–36 weeks of gestation should be discussed with the woman.

8.1.13. Delivery Visit

Delivery may occur at a hospital other than clinical site in the event of an emergency. For the Delivery Visit, the investigator/clinical coordinator can visit the delivery hospital or the participant can visit the investigative site after delivery. Whenever possible, every effort should be made to collect all assessments specified in the SoA (Table 1) for this visit, especially the PRO/quality-of-life assessments, within 24 hours after delivery.

8.2. Efficacy Assessments

8.2.1. Serum Bile Acids

Samples for sBA determinations will be collected at times shown in the SoA (Table 1). The samples will be frozen and shipped to the designated laboratory for analysis for total sBA, as needed. Sample collection, processing, and shipping instructions will be provided to the site separately.

8.2.2. Adult ItchRO

The Adult ItchRO (Appendix 4) is an 11-point NRS measurement of worst itch severity ranging from 0=no itch to 10=worst possible itch, rated over the past 24 hours and completed daily in an eDiary within a prespecified time window.

8.2.3. Composite Adverse Perinatal Outcomes

The composite adverse perinatal outcomes will comprise the following:

- Perinatal death, defined as in utero fetal death after randomization or known neonatal death up to 28 days after birth
- Spontaneous preterm delivery, defined as delivery at <37 weeks' gestational age following spontaneous onset of preterm labor or PPROM
- Iatrogenic preterm delivery attributable to ICP or ICP-related complications, defined as delivery at <37 weeks' gestational age resulting from medical intervention (i.e., augmentation/induction of labor or cesarean delivery without prior spontaneous onset of preterm labor or PPROM with one or more of the following as the indication for delivery):
  - Diagnosis of ICP with or without suspected fetal compromise
  - Persistently elevated/increasing sBA level
  - Worsening hepatic function
  - Intolerable maternal ICP symptoms
- Neonatal unit admission for ≥12 hours from birth until hospital discharge for one or more of the following indications:
  - Respiratory insufficiency/failure with requirement for oxygen supplementation
  - Noninvasive or invasive respiratory support
  - Hemodynamic instability or shock requiring clinical intervention
  - Metabolic acidosis or signs of asphyxia
  - Proven infection
  - Hypoglycemia or feeding problems requiring IV fluids or tube feeding
  - Meconium-stained amniotic fluid and/or its sequelae (e.g., meconium aspiration, etc.)

Data on outcomes described in the SoA (Table 1) under "Perinatal outcomes assessment" shall be collected in the eCRFs.

8.2.4. 5-D Itch Scale

The 5-D Itch scale is a 5-item measure of 5 dimensions of itching (duration, degree [intensity], direction [degree of change], disability [impact], and distribution [location]). See the SoA (Table 1) and Appendix 5 for time points and details.

8.2.5. Clinician Scratch Scale

The CSS is a clinician assessment of itch severity, and to the extent possible, should be made by the same investigator or designee during study visits. The assessment focuses on visible damage to the skin as a result of scratching, as observed by the clinician. Clinicians rate a participant's skin damage due to itch using 5 rating options, ranging from None to Cutaneous mutilation, hemorrhage, and scarring evident. The CSS will be used as a co-validator in the assessment of the convergent validity of the Adult ItchRO scores. See the SoA (Table 1) and Appendix 6 for time points and details.

8.2.6. EuroQoL 5-Dimension 5-Level

The EQ-5D-5L is a widely used standardized measure of health status that provides a descriptive profile and single index value to appraise a respondents' health status (Herdman et al. 2011; Oppe et al. 2014). The measure was designed for both clinical and economic appraisal and has been validated in numerous clinical populations (Cella et al. 2012). It uses a recall period of "Today." The EQ-5D-5L comprises 5 dimensions: Mobility, Self-care, Usual Activities, Pain/Discomfort, and Anxiety/Depression. These dimensions are measured using a 5-point rating scale ranging from no problems to extreme problems (e.g., I am extremely anxious or depressed). Developers of the measure deliberately describe the rating scale for the measure as noncardinal with no arithmetic properties. Responses to the 5 dimensions are used to compute a single utility index score ranging from 0.0 to 1.0, which represents the general health status of the individual. The EQ-5D-5L also contains a visual analog scale to assess the participant's overall health. The EQ-5D-5L will be used in exploratory descriptive analyses. The EQ-5D-5L will be used as a co-validator in the assessment of the convergent validity of the Adult ItchRO scores.

8.2.7. PROMIS SF v1.0-Fatigue 7a

The PROMIS SF v1.0-Fatigue 7a is the original 7-item short form of the PROMIS Fatigue measure. See the SoA (Table 1) and Appendix 7 for time points and details.

8.2.8. PROMIS SF v1.0-Sleep Disturbance

The PROMIS SF v1.0-Sleep Disturbance in an 8-item measure that assesses quality of sleep, sleep depth, and restoration associated with sleep (Yu et al. 2012). See the SoA (Table 1) and Appendix 7 for time points and details.

8.2.9. Patient Impression of Severity-Itch

The PIS-Itch is a modified version of commonly used impression of change scale designed for self-administration. See the SoA (Table 1) and Appendix 8 for time points and details.

8.2.10. Patient Global Impression of Change-Itch

The PGIC-Itch was designed to assess change in itching after being treated with study drug from the specified time point compared with baseline. See the SoA (Table 1) and Appendix 9 for time points and details.

8.2.11. Exploratory Markers of Bile Acid Synthesis, Inflammation, Pruritogens

Serum 7αC4 and FGF-19 will be evaluated as markers of bile acid synthesis. Samples will be collected at the times shown in the SoA (Table 1). Sample collection, processing, and shipping instructions will be provided to the site separately.

Additional biomarkers of inflammation (GLP-1) and pruritogens (autotaxin, progesterone sulfate) will also be evaluated and collected at times shown in the SoA.

8.3. Adverse Event Collection

At each study visit, participants will be questioned in a general way to ascertain whether AEs have occurred since the previous visit (e.g., "Have you had any health problems since your last visit?"). Refer to Section 9 for additional information.

The investigator and/or designees are responsible for detecting, documenting, and reporting events that meet the AE/SAE/AESI/ECI reporting criteria described in Sections 9.2.1 and 9.2.6.

8.3.1. Time Period for Collection

All SAEs will be collected from the signing of the ICF until whichever of the following time points comes last: the F2 or up to 30 days after date of discharge from hospital for mother or for the baby, respectively, as specified in the SoA (Table 1).

All AEs will be collected from first dose of study drug until whichever of the following time points comes last: the F2, or up to 30 days after date of discharge from hospital for mother or for the baby, as specified in the SoA (Table 1).

All SAEs/AESIs will be reported to the sponsor or designee within 24 hours of the site staff or personnel awareness.

Adverse perinatal events that are included in the key secondary composite endpoint should not be captured as AEs or SAEs because they will already be captured as endpoints for the purposes of efficacy assessment. Refer to Sections 3.2 and 3.3 for details.

Medical occurrences that begin before the start of study drug but after obtaining informed consent will be recorded in the medical history/current medical conditions eCRF and not the AE eCRF unless they meet seriousness criteria.

8.4. Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Table 1).

8.4.1. Perinatal Assessment

The following data and/or assessments will be collected on or after the delivery visit per the SoA (Table 1); the time period for collection of newborn data is as outlined in Section 8.3.1.

- Gestational age at delivery
- Mode of delivery (e.g., vaginal, operative vaginal, cesarean; and iatrogenic versus spontaneous)
- Indication(s) for delivery, if iatrogenic
- Maternal estimated blood loss at delivery
- Apgar score at 5 minutes of life
- Birth weight of infant
- Placental weight
- Umbilical cord assessments, when possible (see Section 8.4.8 for details on order of collections)
- Maternal and fetal (umbilical cord, when possible) volixibat concentrations
- Presence/absence of meconium-stained amniotic fluid
- Neonatal admission diagnoses, treatment details, duration of stay including NICU data if applicable

8.4.2. Fetal Ultrasound

At screening or baseline and every 4 weeks while on study drug, a fetal ultrasound should be performed to exclude evidence of significant intrauterine fetal growth restriction or placental insufficiency. This ultrasound should include umbilical artery dopplers if indicated for estimated fetal weight $<10^{th}$ percentile or for other obstetric indications. Ultrasounds shall be performed and read locally.

Results should be reviewed by the investigator or qualified delegate within 1 business day. If the ultrasound demonstrates evidence suggesting new-onset intrauterine growth restriction (e.g., new finding of estimated fetal weight $<10^{th}$ percentile, or estimated fetal weight known to be $<10^{th}$ percentile before dosing with a decrease to $<3^{rd}$ percentile while on study drug) or evidence of new onset placental insufficiency (e.g., new onset of absent or reversed end diastolic flow), the participant should be managed according to local obstetric standard of care/best practices and the medical monitor should be notified.

8.4.3. Antepartum Fetal Monitoring

To ensure fetal well-being, antepartum fetal monitoring will be performed before and after the first dose of study drug; thereafter, fetal monitoring will be continued at the investigator's discretion or for local standard-of-care obstetric indications. The following are acceptable options for fetal monitoring:

- NST/CTG

- BPP
- Fetal heart rate auscultation using Doppler device, ultrasound, or fetal stethoscope (i.e., fetoscope), only if early gestational age precludes a technically adequate NST/CTG or BPP (e.g., gestational age <24 weeks)

Results of the monitoring assessment should be reviewed by the investigator or qualified delegate prior to the participant leaving the clinic. Participants with non-reassuring fetal assessments should be managed according to local obstetric standard of care/best practices.

8.4.4. Physical Examination

A complete physical examination will be performed by the investigator or designee and will include an assessment of the following body systems: general appearance; skin; head, eyes, ears, nose, and throat; spine, neck, thyroid; musculoskeletal; cardiovascular; respiratory; neurological; and abdomen (including liver and kidneys). Height (at screening only) and weight (on days as specified on the SoA [Table 1]) will also be measured and recorded. A brief physical examination will be a symptom-directed examination focused on changes from the last examination. Investigators should pay special attention to clinical signs related to previous serious illnesses.

Abnormalities identified during screening will be documented in the participant's source documents, on the medical history eCRF, and/or the AE eCRF (if the AE meets seriousness criteria). New findings or changes after the first dose of study drug will be captured as AEs on the AE eCRF page, as deemed by the investigator.

Height and weight at the screening visit and weight upon discharge will be recorded. Height should be measured in centimeters and weight should be measured in kilograms. Measurements should be taken in light clothing and stocking feet (without shoes) with empty pockets. The participant's height should be recorded to the nearest centimeter and weight should be recorded to the nearest 0.1 kg. Height and weight will be used to calculate body mass index (BMI) through use of the following formula:

$$\text{BMI}=\text{weight (kg)}/(\text{height [m]})^2$$

8.4.5. Vital Signs

Vital signs will be taken in the semi-recumbent position after at least 5 minutes of rest and will include systolic and diastolic blood pressure and pulse at all time points. Vital signs may be repeated at the screening visit as needed.

8.4.6. Fetal Heart Rate Confirmation

Confirmation of fetal cardiac activity via auscultation with a Doppler device, ultrasound, or fetal stethoscope (i.e., fetoscope) should be performed at each visit while the participant is pregnant. Participants with non-reassuring fetal assessments should be managed according to local obstetric standard of care/best practices.

8.4.7. Clinical Laboratory Evaluations

Refer to Appendix 1 for the list of clinical laboratory tests to be performed and to the SoA (Table 1) for the timing and frequency. The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with an underlying disease.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 7 (±2) days after the last dose of study drug should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.

If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

All protocol-required laboratory assessments, as defined in Appendix 1, must be conducted in accordance with the SoA (Table 1).

If laboratory values from non-protocol–specified laboratory assessments performed at a local laboratory require a change in participant management or are considered clinically significant by the investigator (e.g., SAE or AE), then the results must be recorded in the eCRF. The investigators will maintain a copy of the reference ranges (with the record of the reference ranges) for the local laboratory(ies) used.

All clinical laboratory assays will be performed according to the laboratory's normal procedures. Reference ranges are to be supplied by the laboratory and will be used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Abnormal clinical laboratory values that are unexpected or not explained by the participant's clinical condition may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

Sample reserves may be obtained by aliquoting samples already drawn for scheduled assessments per the SoA (Table 1); please refer to the study laboratory manual for additional details and instructions. These samples can be stored up to 5 years following the completion of the study and used for additional safety assessments, measure drug levels, potential new biochemical markers, and/or to replace any missing or discarded samples.

8.4.8. Umbilical Cord Assessment

An umbilical cord sample will be obtained at delivery, when possible, for the (potential) assessments of:

- Umbilical artery pH
- Volixibat plasma levels
- sBA

- Total cholesterol
- LDL
- Glucose

If obtained, umbilical artery pH sampling should be performed first, followed by sampling for volixibat plasma levels. Thereafter, samples for sBA, total cholesterol, LDL cholesterol, and glucose may be obtained in any order.

8.4.9. Drug and Alcohol Screen

A urine screen for alcohol and drugs of abuse will be performed at screening as described in the SoA (Table 1). Additional drug and alcohol screens may be performed at the investigator's discretion.

Urine samples are to be tested for ethanol, amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiate metabolite, and phencyclidine.

Results of urine drug and alcohol screens will be reviewed and verified by the study monitor (CRA) but will not be collected in the eCRF database. Any positive result for drugs of abuse or alcohol at the screening visit will exclude the participant from further participation in the study.

8.4.10. Serology Screen

At the screening visit, a blood sample will be drawn to test for the presence of HIV, HBsAg, and HCV antibodies. The test results must be confirmed as negative before enrollment in the study. If a test result is positive, the participant will be excluded from entering the study. Results of the virology screen will be reviewed and verified by the study monitor (CRA) but will not be collected in the eCRF.

8.4.11. Coagulation Panel

The coagulation panel is listed in Appendix 1. A blood sample will be drawn at the time points described in the SoA (Table 1).

8.4.12. Lipid Panel

A blood sample will be drawn for a full lipid panel at the time points described in the SoA (Table 1).

8.4.13. Glycated Albumin Evaluation

A blood sample will be drawn for glycated albumin, a biomarker of glucose metabolism, at the time points described in the SoA (Table 1).

8.4.14. Fat-Soluble Vitamin Evaluation

Fat-soluble vitamin levels (vitamins A, D, and E) will be assessed at the time points indicated in the SoA (Table 1). It is important to protect the sample collection tube from light when drawing samples for these analytes.

8.4.15. Electrocardiograms

At or after the screening visit, local ECGs may be performed, if clinically indicated for evaluation of symptoms.

8.5. Pharmacokinetics

Plasma samples will be collected for measurement of maternal plasma and umbilical cord plasma (when possible) concentrations of volixibat as specified in the SoA (Table 1).

The actual date and time (24-hour clock time) of each sample will be recorded. Sample collection, processing, and shipping instructions will be provided to the site separately in the study laboratory manual.

Each plasma sample will be divided into 2 aliquots (1 each for initial analysis and a back-up). Samples collected for analyses of volixibat plasma concentration may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.

Genetic analyses will not be performed on these plasma samples unless consent for this was included in the informed consent. Participant confidentiality will be maintained.

8.6. Pharmacodynamics

Blood samples will be collected for measurement of sBA at time points specified in the SoA (Table 1). The actual collection times will be reported in the eCRF. The samples will be frozen and shipped to the designated laboratory for analysis of total sBA, as needed. Sample collection, processing, and shipping instructions will be provided to the site separately in the study laboratory manual.

8.7. Biomarkers

Collection of samples for other biomarker research is also part of this study; please see the study laboratory manual, SoA (Table 1), and Section 8.2.11 for details.

8.8. Genetics

Sample(s) (maternal and/or umbilical cord) for DNA isolation may be collected from participants who have consented to participate in the genetic analysis component of the study. Participation is optional. Participants who do not wish to participate in the genetic research may still participate in the study.

In the event of DNA extraction failure, a replacement genetic blood sample may be requested from the participant. Signed informed consent will be required to obtain a replacement sample unless it was included in the original consent.

See Appendix 2 for information regarding genetic research. Details on processes for collection and shipment and destruction of these samples will be specified in the study laboratory manual prior to study initiation.

8.9. Healthcare Utilization

Healthcare utilization data associated with medical encounters for the mother and for the neonate will be collected in the eCRF for all participants, as specified in the SoA (Table 1). Protocol-mandated procedures, tests, and encounters are excluded.

The data collected may be used to conduct exploratory economic analyses and will include the following:

- Number and duration of medical care encounters, including surgeries, and other selected procedures (inpatient and outpatient)
- Dates and duration of hospitalization (total days or length of stay, including duration by wards [e.g., intensive care unit])
- Number and type of diagnostic and therapeutic tests and procedures
- Outpatient medical encounters and treatments (including physician or emergency department visits, tests and procedures, and medications)

9. SAFETY REPORTING

9.1. Safety Parameters and Definitions

9.1.1. Adverse Events

An AE is any untoward medical occurrence in a patient or clinical study participant or the newborn, temporally associated with the use of study drug, whether or not considered related to the study drug. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding or vital sign), symptom, or disease (new or exacerbated) temporally associated with the use of study drug. For the AE collection period, see the SoA (Table 1) and Section 8.3.1.

This includes the following:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent preexisting condition including either an increase in frequency and/or intensity of the condition
- New conditions detected or diagnosed after study drug administration even though it may have been present before the start of the study

The following events are NOT considered an AE:

- Any clinically significant abnormal laboratory findings, vital signs or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition

- An investigational abnormality (e.g., laboratory parameter, vital sign, or ECG) unless the abnormality is considered clinically significant by the investigator based on at least 1 of the following criteria:
  - Induces clinical signs or symptoms
  - Requires active intervention (including referring a participant to a specialist)
  - Requires interruption or discontinuation of study drug
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. Adverse perinatal events that are included in the key secondary composite endpoint should not be captured as AEs or SAEs because they will already be captured as endpoints for the purposes of efficacy assessment.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE
- Elective procedures and preplanned interventions for preexisting conditions that did not worsen since the start of the study
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital)
- Anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) present or detected at the start of the study that do not worsen

9.1.2. Serious Adverse Events

If an event is not an AE per definition above, then it cannot be an SAE, even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease). An SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death
- Is life-threatening (the term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization

  Hospitalization signifies that the participant has been detained (usually involving at least a 24-hour stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline is not considered an AE.

Hospitalization for delivery is not considered an SAE unless there was prolongation of the hospitalization beyond the normally expected duration for vaginal or cesarean birth; in such situations, the diagnosis responsible for the prolongation of hospitalization should be considered the SAE.

- Results in persistent disability/incapacity

  A substantial disruption of a person's ability to conduct normal life functions

  This definition is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

- Is a congenital anomaly/birth defect

- Other situations (Important Medical Events)

  Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency department or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

9.2. Reporting and Follow-Up of AEs and/or SAEs

9.2.1. AE and SAE Reporting

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator should report all relevant AESI/SAE information within 24 hours of site personnel awareness of the event following the instructions provided by the sponsor.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by the sponsor or designee. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the sponsor or designee.

- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

9.2.2. Assessment of Intensity/Severity

The investigator will make an assessment of the maximum intensity/severity for each AE and SAE according to the National Cancer Institute CTCAE, version 5.0.

An event is defined as "serious" when it meets at least one of the predefined outcomes as described in the definition of an SAE, **not** when it is rated as severe.

Should a participant experience any AE not listed in the CTCAE, the following grading system should be used to assess severity:

- **Grade 1**: Mild; asymptomatic or mild symptoms OR clinical or diagnostic observations only OR intervention not indicated.
- **Grade 2**: Moderate OR minimal, local, or noninvasive intervention indicated OR limiting age-appropriate instrumental activities of daily living.
- **Grade 3**: Severe or medically significant but not immediately life-threatening OR hospitalization or prolongation of hospitalization indicated OR disabling OR limiting self-care activities of daily living.
- **Grade 4**: Life-threatening consequences OR urgent intervention indicated.
- **Grade 5**: Death

9.2.3. Assessment of Causality

- The investigator is obligated to assess the relationship (related or not related) between study drug and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship rather than that a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study drug administration will be considered and investigated.
- The investigator will also consult the IB for the assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he or she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to the sponsor or designee. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the sponsor or designee.

- The investigator may change his or her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The investigator and sponsor causality assessments are used to determine whether an event meets the expedited safety reporting requirements.

9.2.4. Reference Safety Information

The Reference Safety Information within the Volixibat IB will be used for expectedness assessment for expedited safety reporting of SAEs.

9.2.5. Expedited Safety Reporting

The sponsor will comply with country-specific regulatory requirements relating to expedited safety reporting to regulatory authorities, institutional review boards (IRBs)/independent ethics committees (IECs), and investigators.

An investigator who receives safety information (e.g., expedited safety report, periodic safety summaries, etc.) from the sponsor may need to notify the local IRB/IEC, according to local requirements.

9.2.6. Adverse Events of Special Interest

For this study, AESIs will include symptomatic cholelithiasis and threatened preterm labor; such events must be reported to the sponsor within 24 hours of site personnel awareness of the event, regardless of regulatory seriousness criteria or causality. Specific reporting instructions will be included in the eCRF completion guidelines.

9.2.7. Events of Clinical Interest

ECIs will include CTCAE Grade ≥3 diarrhea, CTCAE Grade ≥3 abdominal pain, CTCAE Grade ≥3 transaminase increases, and fat-soluble vitamin deficiency; these will be subject to specialized, targeted data collections. Please refer to Appendix 3 for algorithms to assess transaminase increases and/or potential DILI events.

Additional ECIs may be identified during the course of the study.

Specifics regarding events to be designated as ECIs, along with reporting instructions/timelines, will be included in the eCRF completion guidelines. ECIs that meet seriousness criteria shall be subject to expedited reporting as outlined in Sections 9.2.1 and 9.2.5.

9.2.8. Overdose and Special Reporting Situations

Overdose is defined as intentional or unintentional intake of a dose of investigational product higher than the protocol-prescribed dose. Treatment of overdose with volixibat would be consistent of general supportive measures, if applicable, including hydration and monitoring for fat-soluble vitamin deficiencies. In the event of an overdose, the investigator should contact the medical monitor within 24 hours of site personnel awareness of the event. The investigator must closely monitor the participant for any AE/SAEs and laboratory abnormalities for at least 24 hours.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the medical monitor based on the clinical evaluation of the participant. The sponsor does not recommend specific treatment for an overdose.

Abuse, misuse, overdose, or medication errors are not considered AEs on their own; however, they must be reported to the sponsor whether or not they result in an AE/SAE as described in Section 9.2.1. All AEs associated with abuse, misuse, overdose, or medication errors should be reported as per the standard process for reporting any AE. The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet >1 category.

- Abuse: Persistent or sporadic intentional intake of investigational product when used for a nonmedical purpose (e.g., to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society
- Misuse: Intentional use of investigational product other than as directed or indicated at any dose (Note: This includes a situation where the investigational product is not used as directed at the dose prescribed by the protocol)
- Overdose: Intentional or unintentional intake of a dose of investigational product higher than the protocol-prescribed dose
- Medication Error: An error made in prescribing, dispensing, administration, and/or use of an investigational product. For studies, medication errors are reportable to the sponsor only as defined below.
    - Cases of participants missing doses of the investigational product are not considered reportable as medication errors.
    - Medication errors should be collected/reported for all products under investigation. The administration and/or use of the unassigned treatment is/are always reportable as a medication error.
    - The administration and/or use of an expired investigational product should be considered as a reportable medication error.

9.2.8.1. Disease Progression

Disease progression itself (i.e., worsening of ICP) is not considered an AE unless it is considered atypical in presentation and/or considered to be related to the study drug. Signs or symptoms of disease progression do not need to be reported as AEs/SAEs unless there is uncertainty as to their cause, in which case they may be reported as AEs.

9.2.8.2. Liver Safety Monitoring

Please refer to Appendix 3 for algorithms to assess liver safety and potential DILI events.

9.2.9. Lactation/Exposure during Breastfeeding

Mothers who are breastfeeding at screening or planning to breastfeed at any time starting with the screening visit through completion of study drug with volixibat are excluded from study participation.

The potential for volixibat to be excreted in human milk has not been studied at this time. However, given that volixibat is minimally absorbed and only sporadically detectable in plasma (most concentrations below the LLOQ), after single doses up to 300 mg and multiple doses up to 80 mg BID with no observed accumulation, identification of detectable volixibat concentrations in human milk would be unlikely at the doses intended for study.

The protocol-specified treatment period for volixibat ends with delivery. There are no contraindications to initiation of breastfeeding the neonate immediately upon delivery.

9.2.10. Follow-Up

After the initial AE/SAE/AESI/ECI report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All events will be followed until the event resolves, returns to baseline, stabilizes, is otherwise explained, no further improvement is anticipated based on the medical judgement of the treating physician, or the participant is lost to follow-up.

Investigators are not obligated to actively seek AEs or SAEs after study participants exit the study. However, if the investigator learns of any SAEs that occurred in a participant after study exit and he or she considers the event to be reasonably related to the study drug, the investigator must notify the sponsor within 24 hours after awareness.

10. STATISTICAL CONSIDERATIONS

All predefined statistical analyses for the final analysis will be performed after the database is locked. All statistical analyses will be performed using SAS software (SAS Institute, Cary, NC, USA) version 9.4 or higher.

The statistical analysis plan (SAP) will provide the comprehensive statistical methods and definitions for the analysis of the efficacy and safety data, as well as describe the approaches to be taken for summarizing other study information, such as participant disposition, demographic and baseline characteristics, medical and disease history, study medication exposure, and prior medications. All study data will be presented in participant listings.

The study has 2 separate, self-standing parts, and each will have its own separate analyses. Of note, a pooled safety analysis combining Parts 1 and 2 may be considered, whereas Part 1will not be combined in any of the efficacy analyses. Additionally, efficacy analyses within Part 1 will be summarized separately based on UDCA usage at baseline.

All inferential statistical tests will be 2-sided. Summary statistics for continuous variables will include mean, median, standard deviation, SEM, minimum, maximum, and quartiles. Summary of categorical variables will include sample size, number of occurrences, and percentages (95% CIs will be provided where appropriate).

The SAP will be finalized prior to unblinding to preserve the integrity of the statistical analysis and study conclusions.

10.1. Sample Size Determination

Part 1 of the study is designed for estimation without formal statistical testing; hence, there are no formal power considerations. For example, with 20 participants per group and assuming a standard deviation of 25 for each group, a 95% CI for the mean for each volixibat arm would have a half width of 11.

For the power calculations for Part 2, it is assumed that only a single dose will be selected at the end of Part 1, and a 2-sided alpha 0.05 will be used to test whether there is a difference between the 2 groups. Thus, for Part 2, a sample size of 100 participants per arm has 80% power to detect mean difference of 10 with standard deviation of 25. Power was calculated using SAS, version 9.4.

10.2. Population for Analyses

For purposes of analysis, the populations are defined in Table 3.

Table 3 Analyses Populations

Population	Description
Enrolled	All participants who sign the ICF
ITT	All randomized participants
mITT	All randomized participants who receive at least 1 dose of study drug (partial or complete). Participants will be analyzed according to the treatment to which they were randomized.
PP	All participants in the mITT population who do not experience any major protocol violations
Safety	All participants who receive at least 1 dose of study drug. Participants will be classified based upon the treatment they actually receive.

ICF=informed consent form; ITT=intent-to-treat; mITT=modified intent-to-treat; PP=per-protocol.

10.3. Participant Disposition, Demographics, and Baseline Characteristics

Participant disposition, demographic and baseline characteristics, disease history, prior medications, treatment exposure, and compliance will be summarized by treatment group. Study completion status and reasons for discontinuation will also be displayed. The safety population will be used for these summaries. Medical and surgical history and protocol violations/deviations will be presented in participant listings.

10.4. Statistical Analyses

Data from Part 1 will be included in the overall safety/tolerability summaries; however, participants in Part 1 will not be combined with those from Part 2 for the statistical inference performed in Part 2. Sampling to assess plasma exposures to volixibat may be discontinued in Part 2 pending results in Part 1.

10.4.1. Efficacy Analyses

10.4.1.1. Primary Efficacy Endpoint

For Part 2, the primary efficacy endpoint analysis is defined as the mean change in total sBA concentration from baseline to Week 3.

Primary Estimand for Primary Efficacy Endpoint

The primary estimand efficacy endpoint is to evaluate the superior efficacy of volixibat versus placebo for the reduction of elevated sBA concentrations in participants with ICP. The primary comparison of interest is the mean change in sBA concentration comparing baseline with Week 3. The primary comparison will be made regardless of adherence to randomized treatment, treatment modification, or treatment termination and irrespective of any changes in or initiations of allowed concomitant therapies, while assuming that prohibited medications are not available.

The corresponding primary estimand is described by the following 5 attributes:

- Treatments: The randomized treatment administered BID until delivery (with at least 1 dose), regardless of adherence to randomized treatment, treatment modification, or treatment termination
- Population: Participants with ICP who have an sBA concentration ≥20 µmol/L and an average daily score of ≥4 on the Adult ItchRO during the screening period. Further population criteria details are provided in Sections 5.1 and 5.2.
- Endpoint: Change in the sBA concentration comparing baseline with Week 3
- Population-level summary: Difference in the mean change in the sBA concentration
- Intercurrent events:
  - Change in or administration of any allowed concomitant medication will be handled using a treatment policy strategy.
  - Administration of any prohibited concomitant medication will be handled using a hypothetical strategy assuming that the prohibited medications are not available and hence were not taken, so as to assess the treatment effect for the randomized treatment groups on top of allowed concomitant medication.
  - Discontinuation of randomized treatment due to all of the following reasons will be handled using a treatment policy strategy:
    - Lack of efficacy
    - Safety concerns
    - Lack of compliance to study treatment
  - Delivery prior to 3 weeks of treatment will be handled using a hypothetical strategy, assuming that delivery has not occurred. Few early deliveries are expected prior to Week 3. Participants with sBA <100 are not likely to give birth prior to 39 weeks, which is at least 3 weeks after the first dose date based on

eligibility criteria (see Section 8.1.12). Participants with sBA >100 at 34 weeks would have been randomized at least a week earlier (i.e., 33 weeks) because a planned birth within 7 days of randomization is exclusionary. It is anticipated that these participants would still be able to remain on treatment for 3 weeks before giving birth.

- Perinatal death prior to 3 weeks of treatment will be handled using a hypothetical strategy, assuming perinatal death has not occurred. Very few, if any, perinatal deaths are expected prior to Week 3.
- Discontinuation of randomized treatment due to other reasons (including loss to follow-up) will be handled using a hypothetical strategy, assuming that randomized treatment had not been discontinued.
- Liver transplant or death: Neither of these are expected in the study. In the unlikely event of this happening, they will be treated using a composite strategy, assuming worse case for all subsequent assessments.

Primary Analysis for Primary Efficacy Endpoint

The primary analysis on the primary efficacy endpoint will be conducted in Part 2 using the modified intent-to-treat (mITT) population. A restricted maximum likelihood (REML)–based mixed-effects model for repeated measures (MMRM) will be used as the primary analysis method. The repeated measures include weekly postbaseline visits through Week 3, with the change from baseline in total sBA as the dependent variable. The MMRM model will include the fixed, continuous effects of baseline sBA and gestational age, fixed categorical effects of presence/absence of gestational diabetes, treatment group, visit, and treatment group-by-visit interaction and participant as a random effect.

The unstructured variance/covariance matrix will be used to model the variances and covariances for the time points included in the model. The unstructured variance/covariance does not impose any restrictions on the pattern of the matrix elements. The use of an endpoint 3 weeks after baseline should result in minimal amounts of missing data. Every attempt (e.g., relaxing the convergence criteria, increasing the iteration limit, choosing reasonable starting values for the estimates) will be made to ensure convergence using the unstructured modeling of within-participant correlations. However, if the numerical algorithm for estimation of the mixed model fails to converge, the following variance/covariance matrix structures will be used in the following order: 1) heterogeneous Toeplitz, 2) heterogeneous compound symmetry, and 3) heterogeneous autoregressive of order 1. The first (co)variance structure that does not have a convergence problem will be the one used for the analysis. The Kenward-Roger approximation (1997) will be used to estimate denominator degrees of freedom.

The primary efficacy analysis will compare volixibat and placebo using the contrast (difference in least squares [LS] means) between treatment groups at Week 3. Significance tests will be based on LS means using a 2-sided significance level (2-sided 95% CIs).

The null hypothesis for the primary efficacy endpoint of the equality of volixibat and placebo is:

H_0: mean change in total sBA baseline and Week 3 in the 2 treatment groups are equal

The null hypothesis of equal treatment effect will be rejected if the statistical analysis results in a 2-sided p-value for treatment at Week 3 is ≤0.05. LS means will be calculated for each treatment group for each postbaseline visit in the model. The difference between volixibat and placebo change from baseline in sBA will be estimated, with the corresponding 2-sided 95% CI constructed for each visit through Week 3. The change from baseline LS means with SE, 95% CI for the LS means, p-value for testing if the LS mean is zero, LS mean difference between treatment groups (volixibat minus placebo) with SE, 95% CI for the LS mean difference, and p-value for testing if the treatment LS means are equal will be presented.

The study will be claimed successful if the hypothesis of no treatment effect on the primary efficacy endpoint over Part 2, in the mITT population is rejected at the 0.05 (2-sided) significance level.

Separately, a similar analysis on each of the open-label cohorts will be performed. The analysis will be identical, except that baseline sBA will not be a covariate used within the model.

Rationale for Primary Analysis Method

The MMRM method has been demonstrated extensively as an appropriate choice for the primary analysis in longitudinal confirmatory clinical studies with continuous endpoints (Mallinckrodt et al. 2008). This analysis method, which is from a broader class of direct-likelihood analyses methods, makes use of fully and partially observed data sequences from individual participants by estimating the covariance between data from different time points (Molenberghs and Kenward 2007). Further, it is often useful to implement MMRM using an unstructured approach to modeling both the treatment-by-time means and the (co)variances, leading to what is essentially a multivariate normal model wherein treatment group means at the primary time point are adjusted to reflect both the actual observed data and the projected outcomes from the participants with missing data (Cnaan et al. 1997; Molenberghs et al. 2004; Molenberghs and Kenward 2007).

Despite careful planning and study conduct, the occurrence of missing data cannot be completely eliminated. As a direct likelihood method, the MMRM method is a preferred approach for handling missing data in such designs. MMRM is a full multivariate model in nature that avoids potential bias as a predetermined model and operates in a more general missing at random (MAR) framework (Mallinckrodt et al. 2001). Data are considered MAR if, conditional upon the independent variables in the analytic model, the missingness depends on the observed outcomes of the variable being analyzed but does not depend on the unobserved outcomes of the variable being analyzed. This assumption implies that the behavior of the post-dropout observations can be predicted from the observed variables and therefore that treatment effect can be estimated without bias with use of the observed data (EMA 2010). For studies of missing data in a controlled clinical study setting, MAR is usually considered as a plausible underlying missing mechanism (Molenberghs and Kenward 2007; Siddiqui et al. 2009; Mallinckrodt et al. 2008, 2013). The assumption of MAR is often reasonable because, particularly in longitudinal studies wherein the evolution of treatment effects is assessed by design over time, the observed data and the models used to analyze them can explain much of the missingness (Little and Rubin 1987; Verbeke and Molenberghs 2000). This point may be especially relevant in well-controlled studies, in which extensive efforts are made to observe all outcomes and factors that influence them while participants are following protocol-defined procedures. Thus, longitudinal clinical

studies by their very design aim to reduce the amount of missing not at random (MNAR) data (missingness explained by unobserved responses), thereby increasing the plausibility of MAR (Mallinckrodt et al. 2008).

Sensitivity and Supportive Analysis for Primary Efficacy Endpoint

Sensitivity and supportive analysis will be performed on the primary efficacy endpoint to quantify the possible impact of missing data and to demonstrate the robustness of the conclusions. See below for details about missing data handling, placebo-based imputation, and a tipping-point analysis.

Furthermore, the primary analysis will be repeated using a hypothetical (de jure) estimand where data collected after rescue medication is initiated will be omitted. This can be viewed as addressing a per protocol estimand, or more simply the applicable estimand if all participants completed the first 3 weeks of follow-up without any rescue or without any missing data.

Summary statistics on total sBA by treatment group and visit will also be presented. In addition, a figure with the LS mean ±SE of change from baseline in total sBA will be presented by treatment group and visit.

In addition, a responder analysis (based on change in total sBA) may be considered. The response definition and its appropriate analysis methodology will be outlined in the SAP.

Missing Data

Although the assumption of MAR, as used for the primary efficacy analysis method, is often reasonable in clinical studies, the possibility of MNAR data cannot be ruled out. Therefore, analysis valid under MNAR is desired. Both a MNAR- and MAR-based analyses will be the basis upon which sensitivity of missing data is assessed.

Sensitivity analyses to deal with missing data will use multiple imputation (MI) methods where missing values are imputed individually under both a plausible MNAR and MAR scenario. MI is a simulation-based approach where missing values are replaced using an appropriate stochastic model given the observed data and covariates, creating multiple completed datasets. These completed datasets are then analyzed using standard analysis methods (i.e., MMRM), and the different parameter estimates across the datasets are then combined to produce a unique point estimate and SE taking into account the uncertainty of the imputation process. The following 2 sensitivity analysis models, one based on the standard MAR approach and the other based on the MNAR approach, will be used to examine robustness of the primary analysis results.

Complete details of the MI procedures, including the methodology, number of imputations, and the seed of the pseudorandom number generator used to randomly generate imputations for the missing values, will be prespecified in the SAP prior to unblinding.

Standard MAR Imputation

The MAR imputation model will impute missing values using a regression-based MI model (Little and Yau 1996). For participants with complete data up to a particular visit, a multiple regression model will be fit that includes the outcome at that visit as the dependent variable and observed data (e.g., outcomes at previous visits, treatment, and baseline) as independent variables. Separate models will be similarly constructed for each visit. Using these regression

models, a missing value for a participant at a particular visit will be imputed as a draw from the predictive distribution given the outcomes at previous visits (some possibly imputed), treatment group, etc. This process will be repeated a given number of times (as specified in the SAP), resulting in the same number of complete analysis datasets. The MMRM analyses will be performed separately for each of the completed analysis datasets, and the results will be combined into 1 MI inference (Little and Yau 1996; Schafer 1997). This strategy is appropriate for datasets that have a monotone missing data pattern. If the dataset does not precisely have this pattern, a monotone data augmentation method, as described in the SAP, will be used to impute the small amount of missing data that is required to make the missing data pattern monotone before applying the MI algorithm described above.

Placebo-Based Imputation

The placebo-based imputation analysis model assumes a MNAR mechanism and is within the pattern-mixture model framework. The pattern-mixture model approach models the distribution of a response as the mixture of a distribution of the observed responses and a distribution of the missing responses. Conceptually, pattern mixture models typically assess the outcome variable separately for different groups (patterns) and then combine results across groups for final inference (Mallinckrodt et al. 2008; Ratitch and O'Kelly 2011). The pattern-mixture model approach will be applied in the SAS MI procedure by using the MNAR statement. The MNAR imputation model will specify a subset of observations to derive the imputation model, which is used for pattern imputation based on the placebo group.

The underlying assumption is that a participant on the active treatment with missing data follows the distribution of the placebo responses (i.e., the means and the intra-participant correlations based on the placebo responses will apply). This sensitivity analysis stress-tests the MAR assumption that withdrawals will tend to have efficacy similar to participants who remain in the study in their respective treatment arm. On the contrary, the placebo-based pattern imputation assumes MNAR; that after discontinuation, participants on study drug will tend to have efficacy close to participants on placebo.

The imputations for this model are based on the distribution of placebo group responses over time. That is, an imputation model for the missing observations in the volixibat treatment group is constructed not from the observed data in the active treatment group but rather from the observed data in the placebo treatment group. This model is also the imputation model that is used to impute missing observations in the placebo group.

Tipping Point Analysis

An additional sensitivity analysis using the tipping-point approach will be conducted to assess the robustness of the primary analysis approach. If it is plausible that, for the active treatment group, the distribution of missing primary endpoint responses has a smaller expected reduction than that of the corresponding distribution of the observed primary endpoint responses, the conclusion under the MAR assumption should be examined. It is desired to impose a fixed and definite set of quantities to encapsulate the change in efficacy associated with withdrawal (missing) for the active treatment group and independently for the placebo group. The tipping point MI analysis as described by Ratitch et al. (2013) will be applied. Tipping point analysis is a means of exploring the influence of missingness on the overall conclusion from statistical

inference by positing a wide spectrum of specifications regarding the missingness mechanism (from less pessimistic to more pessimistic). The analysis finds a (tipping) point in this spectrum of specifications, at which conclusions change from being favorable to the experimental treatment to being neutral. After such a tipping point is determined, clinical judgment can be applied as to the plausibility of the specifications underlying this tipping point. The tipping point can be identified while the result is no longer statistically significant. This imputation analysis uses a specified sequence of shift parameters, which adjust the imputed values for observations in the active treatment group and independently for the placebo group.

10.4.1.2. Adjustment for Multiplicity

In order to maintain study-wide Type I error control, an hierarchical testing procedure will be used in the comparisons between volixibat and placebo on the primary and secondary efficacy endpoints in the mITT population. The hierarchical order for testing the null hypotheses is as follows: 1) mean change in total sBA between baseline and Week 3, 2) mean change in Adult ItchRO between baseline and Week 3, and 3) the proportion of participants experiencing a composite perinatal outcome.

The effect of such a procedure is that no confirmatory claims can be based on the endpoint(s) that have a rank lower than that endpoint whose null hypothesis was the first that could not be rejected. Those tests will be considered as exploratory without any formal conclusions drawn about them.

The analysis of Part 1 will be considered separate from Part 2 and no multiplicity control will be made.

10.4.1.3. Secondary Efficacy Endpoints

The secondary efficacy endpoints (see Section 3.2) are designed to evaluate the efficacy of volixibat versus placebo for the treatment of pruritus in participants with ICP as well as composite perinatal outcomes.

The secondary efficacy endpoint for pruritus is defined as the mean change from baseline to Week 3 in the weekly average worst daily itch score as measured by the Adult ItchRO. The worst daily itch score is averaged over the 7 days preceding baseline and over the 7 days preceding each week through Week 3. If >3 days of assessments are missing from a given week, then that weekly average will be set to missing. The analysis of the Adult ItchRO will use an MMRM with a comparable model to that for the primary endpoint. The primary comparison will be made regardless of adherence to randomized treatment, treatment modification, or treatment termination and irrespective of any changes in, or initiations of, allowed concomitant therapies, while assuming prohibited medications are not available and that delivery or perinatal death prior to 3 weeks does not occur.

The attributes for this estimand are the same as for the primary estimand except for the below:

- Endpoint: Change from baseline to Week 3 of the study treatment period in the weekly averaged worse daily itch score as measured by the Adult ItchRO (average of the 7 days preceding baseline and of the 7 days preceding Week 3).
- Population-level summary: Difference in the mean change in the weekly averaged worse daily itch score

The secondary efficacy endpoint for composite perinatal outcomes is defined as the proportion of participants that experience any of the 4 outcomes specified in Section 3.2. The comparison of interest is the proportion of participants meeting the composite outcome. The primary comparison will be made regardless of adherence to randomized treatment, treatment modification, or treatment termination and irrespective of any changes in, or initiations of, allowed concomitant therapies, while assuming prohibited medications are not available.

The attributes for this estimand are the same as for the primary estimand except for the below:

- Treatments: The randomized treatment administered BID until day of delivery (with at least 1 dose), regardless of adherence to randomized treatment, treatment modification, or treatment termination.
- Endpoint: Experiencing one or more of perinatal death, spontaneous preterm delivery, iatrogenic preterm delivery attributed to ICP or ICP-related complications, neonatal unit admission for ≥12 hours from birth until hospital discharge for one or more of predefined indications.
- Population-level summary: Difference between treatment groups in the proportion of participants achieving the endpoint.
- Intercurrent events: Preterm delivery or perinatal death are included as part of the endpoint definition and thus handled using a composite strategy.

For this responder-type endpoint, the number and proportion of participants that are considered a "responder" will be summarized by treatment group at Week 3 and analyzed using a stratified Cochran-Mantel-Haenszel (CMH) test to calculate the p-value for the difference between treatment groups. The p-value from the CMH test will be the basis of the hypothesis testing. Furthermore, as supportive analyses, a Logistic Regression (LR) estimator approach (Ge et al. 2011), as well as a logistic regression model with baseline sBA, gestational age, and presence/absence of gestational diabetes as covariates will be run. The model assumptions of the logistic regression model are not considered a concern because a randomized study has comparable distributions of the treatment groups for covariates and factors for stratification, although they may impact the interpretation of estimated parameters for the covariates. Most importantly, a logistic regression model addresses estimands that are conditional on the covariates in the model, with these estimands sometimes being called participant-specific, and express the extent of efficacy of a treatment that a participant might expect on the basis of her covariates, although they tend to be further from the null for an effective treatment than population average estimand. A randomization-based version of logistic regression will also be considered. It is robust to model assumptions, and it has a population average estimand that is the same as the unadjusted OR for comparing 2 treatments (Zinc and Koch 2012).

An hierarchical testing procedure, as described in Section 10.4.1.2, will be used in the comparisons between volixibat and placebo on the primary and secondary efficacy endpoints for Part 2 in the mITT population using the primary analysis method (as described for the primary endpoint). Sensitivity analyses for the secondary endpoints included within the hierarchical testing procedure will be detailed within the SAP.

All tests will be performed as 2-sided tests at the 0.05 level of significance.

10.4.1.4. Exploratory Efficacy Endpoints

The exploratory efficacy endpoints are defined in Section 3.4. The change in total sBA from Week 3 (primary endpoint) to delivery will be considered, and the difference between treatment groups will be tested using a randomization-based version of rank analysis of covariance (ANCOVA; Stokes et al. 2012). In a similar manner, the change from baseline in total sBA concentration to the time of delivery will also be explored. An additional analysis will be performed that examines the change from baseline to delivery but that averages across the last 2 time points on or prior to delivery. Similar analyses will be performed for Adult ItchRO. Associations between the changes from baseline to Week 3 in total sBA and Adult ItchRO will be assessed using either Pearson's or Spearman's correlation coefficient. "Responder" analyses for composite perinatal outcomes will be performed using baseline sBA and ItchRO and the change at Week 3 values in sBA and ItchRO as covariates and, separately, using the Week 3 values themselves (instead of the change at Week 3).

Change in biomarkers of bile acid synthesis, inflammation, and pruritogens as well as the proportion of participants with sBA <40 μmol/L at the end of the treatment period will also be explored. The change in biomarkers will follow the same approach as other exploratory efficacy endpoints described above. The proportion of participants with sBA <40 μmol/L at the end of the treatment period will be analyzed in the same manner as the secondary efficacy endpoint for composite perinatal outcomes.

Further exploratory efficacy endpoints include continuous and binary endpoints on additional perinatal outcomes. The analysis of continuous endpoints will follow the same approach as other exploratory efficacy endpoints described above. Binary outcomes will be analyzed in the same manner as the secondary efficacy endpoint for composite perinatal outcomes. Additional details will be included within the SAP.

The secondary endpoint of composite perinatal outcomes will also be explored using an ordinal scale, with ranks assigned according to the severity of the outcomes specified within Section 3.2.

Table 4 Perinatal Outcome Severity Scoring

Perinatal Outcome	Score
Perinatal death	4
Spontaneous preterm delivery	3
Iatrogenic preterm delivery attribute to ICP or ICP-related complications	2
Neonatal unit admission for ≥12 hours from birth until hospital discharge for any of the reasons listed in Section 3.2	1
No perinatal outcome	0

ICP=intrahepatic cholestasis of pregnancy.

The score is assigned according to the most severe outcome following the order shown above. If a participant experiences perinatal death, spontaneous preterm delivery, or iatrogenic preterm delivery attributed to ICP or ICP-related complications AND neonatal unit admission for ≥12 hours from birth until hospital discharge for any of the reasons listed in Section 3.2, the score will be adjusted by 0.5. For example, if a participant has spontaneous preterm delivery followed by a neonatal unit admission, the score would be 3+0.5=3.5, where the 3 comes from the spontaneous preterm delivery and the 0.5 comes from the fact that neonatal unit admission occurred. This will be analyzed using a stratified log rank test that provides more weight to the more severe outcomes and will employ a randomization-based covariance adjustment (Zinc and Koch 2012).

Additional, nonefficacy exploratory endpoints are described within Section 10.4.3.

10.4.2. Safety Analyses

All safety analyses will be performed on the safety population, defined as all participants who were randomized and received at least 1 dose of study medication.

For all safety and tolerability analyses, participants will be analyzed by the treatment received and the overall active treatment group. For active treatment, the dose received will be used.

Safety data collected at the baseline visit or the last preceding visit (if not collected at the baseline visit) will be used as the baseline value for safety analyses.

Safety measures include AEs, clinical laboratory values, physical examination findings (including body weight, height, and BMI), vital signs, and concomitant treatment usage. For quantitative variables, descriptive statistics including number of observations, mean, median, standard deviation, minimum, and maximum will be presented for observed and change from baseline values at each study visit. Qualitative variables will be summarized using counts and percentages.

Beyond the endpoints described within Section 3, any additional fetal data collected will only be listed. These include but are not limited to fetal ultrasounds and antepartum fetal monitoring via NST/CTG, BPP, and fetal heart rate auscultation.

All safety and tolerability data will be presented in listings.

10.4.2.1. Adverse Events

In general, TEAEs are defined as AEs that start or deteriorate on or after the first dose of study medication and no later than 7 days following the last dose of study medication. For any participants (i.e., expectant mothers) who die during the study and the date of death is between the date of the first dose of study medication 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 and will be included in the TEAE summaries. All summaries of AEs will be based on TEAEs unless specified otherwise.

The complete list of Preferred Terms for AESIs and ECIs (as described in Sections 9.2.6 and 9.2.7) will be outlined in the SAP and summarized.

AEs will be coded using MedDRA. The number and proportion of participants who experience the event according to MedDRA System Organ Class (SOC) and Preferred Term will be presented by treatment group.

The incidence of all AEs and treatment-related AEs will be tabulated by treatment received. These AEs will be classified by SOC and Preferred Term using MedDRA. AEs will be graded using the CTCAE, Version 5.0, grading scale. For incidence reporting, if a participant reported >1 AE that was coded to the same SOC or Preferred Term, the participant will be counted only once for that specific SOC or Preferred Term. An overview of AEs and TEAEs, both of which include participant incidence of AEs, treatment-related AEs, AEs by severity, AESIs, ECIs, SAEs, deaths, and AEs that lead to discontinuation, will be presented.

Furthermore, exposure-adjusted incidence rates (EAIR) will be provided for each AE reported. For EAIR analyses, the following convention will be used to calculate the denominator (i.e., population-level time at-risk) for these analyses. The population-level time at-risk includes only the time each participant is at risk. The start date of at-risk time is the date of first dose of study drug, and the last date of at-risk time is the start date of the occurrence of the AE or is censored at completion/early termination, whichever is earlier.

While no formal hypothesis testing or margin selection is planned, the risk difference and corresponding exact 95% CI (using the method of Chan and Zhang [1999]) will be presented to compare treatment groups with regards to AESIs and ECIs.

Participant listings of AEs will include the dose of study medication at the onset of the event.

10.4.2.2. Vital Signs and Weight/Height Measurements

Vital signs (temperature, systolic and diastolic blood pressure, heart rate, and respiratory rate), weight, height, and BMI will be summarized descriptively by study visit and treatment group as observed and the change from baseline values.

10.4.2.3. Laboratory Assessments

Clinical safety laboratory values will be measured by a central laboratory and, where grading criteria apply, will be graded according to the CTCAE grading scale. Summary statistics for actual values and for changes from baseline will be tabulated for laboratory results by scheduled visit. Graphs of laboratory values over time may also be produced.

Participants with clinical laboratory values outside of the normal reference range at any postbaseline assessment will be summarized, including by the CTCAE and, if necessary, other grading scales. Shifts from baseline laboratory values will be tabulated. The observed vital sign data, the change from baseline, and those meeting criteria for potentially clinically significantly abnormal criteria for each measurement will be summarized with descriptive statistics.

10.4.2.4. Prior and Concomitant Treatments

Prior and concomitant treatments will be summarized descriptively by treatment group with the number and proportion of participants.

Prior medications will be presented separate from concomitant treatments. Medications that started prior to the first dose of study medication will be considered prior medications regardless

of whether they were stopped prior to the first dose of study medication. Any medications continuing or starting after the first dose of study medication will be considered to be concomitant. If a medication starts prior to the first dose of study medication and continues after the first dose of study medication, it will be considered both prior and concomitant.

Medications will be coded using the World Health Organization Drug Dictionary.

10.4.3. Other Analyses

10.4.3.1. Patient-Reported Outcomes

The effect of volixibat on additional measures of pruritus efficacy and quality of life in adult participants with ICP will be evaluated via the following endpoints:

- Change from baseline in the 5-D Itch Scale
- Change from baseline in CSS
- Change from baseline in EQ-5D-5L
- Change from baseline in PROMIS SF Fatigue 7a
- Change from baseline in PROMIS SF Sleep Disturbance
- Change from baseline in PIS-Itch

Because the PROs are administered only at baseline, Week 3, and end of treatment, the change from baseline will be compared between the 2 groups with use of rank ANCOVA with adjustment for baseline with the Mann-Whitney odds as the estimand. The estimates and CIs can be calculated using the R package sanon (Kawaguchi and Koch 2015).

10.4.3.2. Healthcare Utilization

Healthcare resource utilization variables include the number and duration of medical care encounters, including surgeries and other selected procedures (inpatient and outpatient), the dates and duration of hospitalization (total days or length of stay, including duration by wards [e.g., intensive care unit]), the number and type of diagnostic and therapeutic tests and procedures, and outpatient medical encounters and treatments (including physician or emergency department visits, tests and procedures, and medications). 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.

10.4.3.3. Pharmacokinetic Analyses

For maternal volixibat plasma concentration assessments: Plasma concentration of volixibat near predicted maximum observed concentration (C_{max}) will be obtained during the Week 1 visit, ~3 hours after dosing. Trough plasma levels of volixibat will be obtained during the Week 3 visit before dosing. Contingent upon meaningful exposures of volixibat, PK endpoints may include but are not limited to C_{max}, time to reach C_{max} (T_{max}), and the AUC from time zero to the last measurable time point. Actual dosing and sampling times will be used for calculation of PK parameters. Additional PK parameters may be calculated.

For fetal (umbilical cord) volixibat plasma concentration assessments: Plasma concentration of volixibat in the umbilical cord blood sample obtained at delivery will be collected (when possible) and analyzed.

10.4.4. Interim Analysis

A formal interim analysis will be conducted at the end of Part 1, which will inform sample size for Part 2 as well as dose selection. There will be ongoing data review during Part 1 after every ~20 participants are enrolled in this open-label setting. Data from Part 1 will be included in the overall safety and tolerability summaries but will not be combined with those from Part 2 for the statistical inference performed in Part 2.

11. REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

11.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
- Applicable International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated at the investigator's site.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

11.2. Financial Disclosure

Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

11.3. Informed Consent Process

The investigator or his or her representative will explain the nature of the study to the participant and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center. If required by local laws and/or regulations, paternal consent may also be required on behalf of the fetus.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled into the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be reconsented to the most current version of the ICF during their participation in the study.

The investigator must maintain the original, signed ICF. A copy of the ICF must be provided to the participant.

The ICF will contain a separate section that addresses the use of any remaining mandatory samples for optional exploratory research. The investigator or authorized designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's agreement to allow any remaining specimens to be used for exploratory research. Participants who decline to participate in this optional research will not provide this separate signature but are still eligible for Study VLX-401.

11.4. Data Protection

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information that would make the participant identifiable will not be transferred.

The participant must be informed that her personal study-related data will be used by the sponsor in accordance with local data protection laws. The level of disclosure must also be explained to the participant.

The participant must be informed that her medical records may be examined by Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

11.5. Committee Structure

11.5.1. Independent Data Monitoring Committee

An IDMC will be formed to monitor the study for safety, ethical conduct, and scientific soundness; to review the efficacy interim analyses during the study; and to make recommendations to the sponsor regarding further conduct of the study, including a recommendation regarding whether the study should be continued, revised, or terminated. The IDMC will be comprised of physicians with expertise in the care and interpretation of studies of ICP and similar liver conditions, and a voting clinical statistician with experience in similar indications. The function of the IDMC will be documented in the IDMC Charter, which will be ratified by signature of all voting members. The first IDMC meeting (post-study start) will be held after 12 participants in Part 2 have completed the study; cadence for future meetings, along with AE monitoring specifications and additional detail regarding procedures, will be outlined in the IDMC charter. IDMC minutes and recommendations will be provided to the regulatory authorities upon request.

11.6. Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data.

11.7. Dissemination of Clinical Study Data

In accordance with the US Food and Drug Administration Amendments Act of 2007 and the European Medicines Agency clinical study Directive 2001/20/EC, the sponsor is solely responsible for determining whether the study and its results are participant to the requirements for submission to www.clinicaltrials.gov, www.clinicaltrialsregister.eu, or other local registries. The sponsor will submit the information necessary to fulfill the requirements. By signing the protocol, the investigator agrees to not submit any information about this study or its results to the registries.

11.8. Data Quality Assurance

All participant data relating to the study will be recorded on printed or eCRFs unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.

The investigator must maintain accurate documentation (source data) that supports the information entered into the eCRF.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

The sponsor or designee is responsible for the data management of this study, including quality checking of the data.

The sponsor assumes accountability for actions delegated to other individuals (e.g., CROs).

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator in accord with country-specific requirements for at least 25 years after completion or discontinuation of the study, or at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region for the drug for the indication for which it is being investigated, or at least 2 years after formal discontinuation of clinical development of the investigational product, whatever is the longest. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to and acceptance by the sponsor.

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor may conduct a quality assurance audit. Refer to Section 11.10 for more details regarding the audit process.

11.9. Study Monitoring

Before an investigational site can enter a participant into the study, a representative of the sponsor will perform a site qualification visit to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities with regard to protocol adherence and the responsibilities of the sponsor or its representatives. This will be documented in a clinical study agreement between the sponsor and the investigator.

During the study, a monitor from the sponsor or representative will have regular contacts with the investigational site for the following:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, data are being accurately recorded in the eCRF, and investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the eCRFs with the participant's medical records at the hospital or practice and other records relevant to the study. This will require direct access to all original records for each participant (e.g., clinic charts)

- Record and report any protocol deviations not previously sent to the sponsor or designee
- Confirm that AEs and SAEs have been properly documented in the eCRFs, confirm that any SAEs have been forwarded to the sponsor, and confirm that those SAEs that met criteria for reporting have been forwarded to the IRB/IEC

The sponsor or designee, including medical monitor, will be available between visits if the investigator(s) or other staff need information or advice.

11.10. Audits and Inspections

Authorized representatives of the sponsor, a regulatory authority, an IEC, or IRB may visit the site to perform audits or inspections, including source data verification. The purpose of a sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted and whether data were recorded, analyzed, and accurately reported according to the protocol, ICH GCP guidelines, and any applicable regulatory requirements. The investigator should contact the sponsor immediately if contacted by a regulatory agency about an inspection.

The sponsor will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, participant charts, study source documents, and other records relative to study conduct.

11.11. Institutional Review Board/Independent Ethics Committee

The investigator must obtain IRB/IEC approval for the investigation. Initial IRB/IEC approval and all materials approved by the IRB/IEC for this study, including the participant ICF and recruitment materials, must be maintained by the investigator and made available for inspection.

11.12. Data Handling and Recordkeeping

11.12.1. Retention of Records

The investigator must maintain all documentation relating to the study according to ICH GCP requirements and in accordance with local country-specific regulations and may not be destroyed without written permission from the sponsor. If it becomes necessary for the sponsor or the regulatory authority to review any documentation relating to the study, the investigator must permit access to such records.

11.12.2. Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data reported on the eCRF or entered into the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Current medical records must be available.

11.13. Study and Site Closure

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

Reasons for the early closure of a study site by the sponsor or investigator may include the following:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study drug development

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Appendix 1 Clinical Laboratory Tests

The laboratory tests detailed below will be performed at the times indicated in the schedule of activities (Table 1). Protocol-specific requirements for inclusion or exclusion of participants are detailed in protocol Section 5. Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

This list reflects the required laboratory assessments as of the effective date of this protocol; however, investigators should refer to the most current version of the study laboratory manual for full details of required laboratory assessments.

Investigators must document their review of each laboratory safety report.

Protocol-required serum or plasma clinical laboratory tests include the following:

- Serology (screening only): HIV, hepatitis B surface antigen, hepatitis C virus antibodies
- Hematology: Hemoglobin, neutrophils (absolute), hematocrit, eosinophils (absolute), RBC count, monocytes (absolute), platelets, basophils (absolute), WBC count (total and differential), lymphocytes (absolute)
- Serum chemistry: Sodium, phosphorus, potassium, protein, glucose, bicarbonate or carbon dioxide, BUN, albumin, creatinine, AST, calcium, ALT, chloride, GGT, thyrotropin (TSH), ALP, total bilirubin
- Coagulation panel: PT/INR, APTT
- Fat-soluble vitamins: Vitamin A (serum retinol), vitamin E (serum α-tocopherol), and vitamin D (serum 25-hydroxycholecalciferol)
- Biomarkers: Glycated albumin, lipid panel, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, VLDL cholesterol, 7αC4, GLP-1, autotaxin, FGF-19, progesterone sulfate.
- Umbilical cord blood (when possible): Arterial blood gas (including pH), volixibat concentration (i.e., PK sample), serum bile acids, total cholesterol, low-density lipoprotein cholesterol, glucose

Protocol-required urine laboratory tests include:

- Urine screen for alcohol and drugs of abuse: Amphetamines, cocaine, barbiturates, methadone, benzodiazepines, opiate metabolite, cannabinoids, phencyclidine, ethanol
- Urinalysis analytes (by dipstick; minimum, the microscopic examination will consist of RBCs, WBCs, casts, and bacteria): pH, bilirubin, glucose, nitrites, protein, leukocyte esterase, blood, specific gravity, ketones

Appendix 2 Genetics

Use/Analysis of DNA

Genetic variation may impact a participant's response to therapy and susceptibility to, severity of, and progression of disease. Variable response to therapy may be because of genetic determinants that impact drug absorption, distribution, metabolism, and excretion; mechanism of action of the drug; disease etiology; and/or molecular subtype of the disease being treated. Therefore, where local regulations and institutional review board/institutional ethics committee allow, a blood sample will be collected for DNA analysis from consenting participants.

DNA samples will be used for research related to volixibat or intrahepatic cholestasis of pregnancy (ICP) and related diseases. They may also be used to develop tests/assays, including diagnostic tests related to volixibat and ICP. Genetic research may consist of the analysis of one or more candidate genes or the analysis of genetic markers throughout the genome (as appropriate).

The samples may be analyzed as part of a multi-study assessment of genetic factors involved in the response to volixibat or study drugs of this class to understand study disease or related conditions.

The results of genetic analyses may be reported in the clinical study report or in a separate study summary.

The sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.

The samples will be retained while research on volixibat continues but no longer than 10 years or other period as per local requirements.

Appendix 3 Liver Safety: Suggested Actions and Follow-Up Assessments

The following algorithms should be used to assess for potential drug-induced liver injury event(s). In the event there is any ambiguity about recommended actions according to the following algorithms, or if there is a scenario that is not covered by the existing algorithms, the study medical monitor should be consulted to determine the appropriate study action.

For Participants with Normal Baseline ALT, AST, and Total Bilirubin

Baseline	ALT during Treatment with Study Drug	Other Concurrent Parameters Observed during ALT or AST Elevation	Study Action[a]
ALT, AST, and total bilirubin ≤ ULN	—	Any hepatic decompensation event	Interrupt study drug and inform study medical monitor ASAP; permanent discontinuation of study drug upon confirmation of diagnosis
	ALT >8 × ULN	—	**Interrupt study drug and** discuss as soon as possible with medical monitor; **consider permanent discontinuation of study drug.**
	ALT >5 × ULN for more than 2 weeks	—	
	ALT >3 × ULN	**and one of the following:** total bilirubin >2 × ULN **or** INR >1.5	**Interrupt study drug** and implement **close observation**
	ALT >3 × ULN	**and** clinical symptoms[b]	
	ALT >3 × ULN	—	Repeat testing within 2–3 days. If results not improved, implement **close observation**

ASAP=as soon as possible; INR=international normalized ratio; ULN=upper limit of normal.

[a] Time frames mentioned under "Study Action" (e.g., ASAP, within 2–3 days) shall be based on date of awareness of laboratory results or events driving the study action.

[b] Clinical symptoms include fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%) not otherwise explained by underlying disease or alternative etiology.

For Participants with Abnormal Baseline ALT, AST, or Total Bilirubin

Baseline	ALT during Treatment with Study Drug	Other Concurrent Parameters Observed during Treatment with Study Drug	Study Action[a]
Any ALT, AST, or total bilirubin > ULN	—	Any hepatic decompensation event	**Interrupt study drug** and inform medical monitor ASAP; **permanent discontinuation of study drug upon confirmation of diagnosis.**
	—	Total bilirubin ≥2 × BLM[b]	**Interrupt study drug** and implement **close observation**
ALT or AST > ULN to <2 × ULN	ALT >5 × BLM or reaches 300 U/L (whichever is lower)	—	**Interrupt study drug** and implement **close observation**
	ALT >2 × BLM	**and** any of the following: total bilirubin ≥2 × BLM[b], INR increase by 0.2[b], or clinical symptoms[c]	
	ALT >2 × BLM	**or** total bilirubin between >1.5 and <2 × BLM[b]	Repeat testing within 2–3 days. If results not improved, implement **close observation**
ALT or AST ≥2 x ULN	ALT >2 × BLM or reaches 300 U/L (whichever is lower)	**or** total bilirubin between >1.5 × and <2 × BLM[b]	Repeat testing within 2–3 days. If results do not improve, implement **close observation.**
	ALT >2 × BLM or reaches 300 U/L (whichever is lower)	**and** any of the following: total bilirubin ≥ 2 × BLM[b], INR increase by 0.2[b], **or** clinical symptoms[c]	**Interrupt study drug** and implement **close observation**
	ALT >3 × BLM or reaches 400 U/L (whichever is lower)	—	

BLM=baseline measurement; INR=international normalized ratio; ULN=upper limit of normal.

Note: See section below for more details on study action.

[a] Time frames mentioned under "Study Action" (e.g., ASAP, within 2-3 days) shall be based on date of awareness of laboratory results or events driving the study action.

[b] Please follow the recommended Study Action if the current total bilirubin or INR value is > ULN. If the current total bilirubin or INR value is ≤ ULN, clinical judgment should be exercised prior to implementing the recommended Study Action.

[c] Clinical symptoms include fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%) not otherwise explained by underlying disease or alternative etiology.

Study Actions

Stop study drug:

- Stop study drug immediately, closely observe the participant (see below), and follow until resolution of symptoms or signs.

Interrupt study drug:

- Stop study drug immediately and repeat ALT, AST, total bilirubin, and/or INR within 3 days and closely observe the participant (see below).
- Study drug can be restarted if liver test results return to baseline levels or if an alternate cause for the transaminase elevations is found and addressed based on the judgment of the investigator (Palmer et al. 2020). Rechallenges should be undertaken only with medical monitor approval.

Close observation:

- Repeat liver enzyme and serum bilirubin tests 2 to 3 times weekly. Frequency of repeat testing can be decreased to once a week or less if abnormalities stabilize or the study drug has been discontinued and the participant is asymptomatic.
- Obtain a detailed history of symptoms and prior or concurrent diseases.
- Obtain a history of concomitant drug use (including nonprescription medications and herbal/dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Rule out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; nonalcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease (except ICP).
- Obtain a history of exposure to environmental chemical agents.
- Obtain additional tests to evaluate liver function as appropriate (e.g., INR, direct bilirubin).
- Consider gastroenterology or hepatology consultations.
-

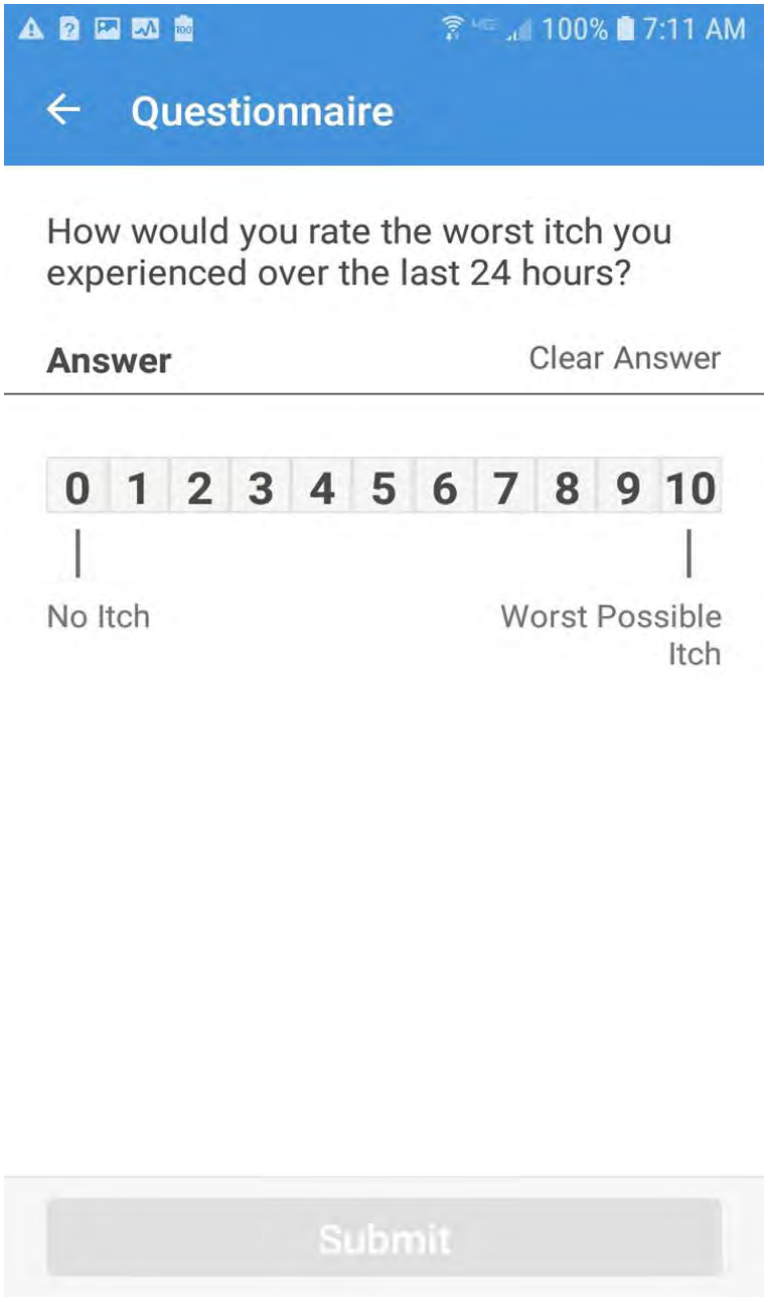
Definition of hepatic decompensation event—presence of any of the following (Palmer et al. 2020):

- Bleeding esophageal varices
- Portal hypertension
- Hepatic encephalopathy
- Ascites

Appendix 4 Adult Itch Reported Outcome

The Adult Itch Reported Outcome (ItchRO) instrument will be completed using an electronic diary (eDiary). The eDiary will be completed once daily using a 24-hour recall period. Each score will have a range from 0 to 10, with higher scores indicating increasing itch severity.

A sample screen shot for the eDiary Adult ItchRO assessment tool is shown in the figure below:



Appendix 5 5-D Itch

The 5-D itch scale was developed for the multidimensional quantification of pruritus that is sensitive to change over time (Elman et al. 2010). The instrument includes domains for duration, degree, direction, disability, and distribution of pruritus. The scores of scores of each of the 5 domains are achieved separately and then summed together to obtain a total 5-D score. The 5-D scores can potentially range between 5 (no pruritus) and 25 (most severe pruritus).

Single-item domain scores (duration, degree, and direction) are equal to the value indicated below the response choice (range, 1–5).

The disability domain includes 4 items that assess the impact of itching on daily activities: sleep, leisure/social activities, housework/errands, and work/school. The score for the disability domain is achieved by taking the highest score on any of the 4 items.

For the distribution domain, the number of affected body parts is tallied (potential sum 0–16), and the sum is sorted into 5 scoring bins.

The 5-D Itch scale for this study will use a 1-week recall period. The 5-D Itch scale may be used to construct a total itch score. Domains 1 through 3 contain only a single-item and require no special scoring to define the domain score. The disability domain is composed of 4 items and is defined as the highest rating observed across the 4 disability items. This produces a disability domain score ranging from 1 to 5. The distribution domain is composed of 16 binary (present/absent) items; these items are summed to create a score defining the count of body areas affected, ranging from 0 to 16. The distribution domain score is then categorized as follows:

5-D Itch Scoring Bins

Distribution Domain Sum (0–16)	Assigned Score
0–2	1
3–5	2
6–10	3
11–13	4
14–16	5

5-D Pruritus Scale

1. **Duration:** During the past week, how many hours a day have you been itching?

Less than 6 hrs/day	6-12 hrs/day	12-18 hrs/day	18-23 hrs/day	All day
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. **Degree:** Please rate the intensity of your itch over the past week.

Not present	Mild	Moderate	Severe	Unbearable
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. **Direction:** Over the past week has your itching gotten better or worse compared to the previous month?

Completely resolved	Much better, but still present	Little bit better, but still present	Unchanged	Getting worse
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

4. **Disability:** Rate the impact of your itching on the following activities over the last week.

	Never affects sleep	Occasionally delays falling asleep	Frequently delays falling asleep	Delays falling asleep and occasionally wakes me up at night	Delays falling asleep and frequently wakes me up at night
Sleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

	N/A	Never affects this activity	Rarely affects this activity	Occasionally affects this activity	Frequently affects this activity	Always affects this activity
Leisure/ Social	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Housework/ Errands	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Work/School	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. **Distribution:** Mark whether itching has been present in the following parts of your body over the last week. If a body part is not listed, choose the one that is closest anatomically.

	Present		Present
Head/Scalp	☐	Soles	☐
Face	☐	Palms	☐
Chest	☐	Tops of Hands/Fingers	☐
Abdomen	☐	Forearms	☐
Back	☐	Upper Arms	☐
Buttocks	☐	Groin	☐
Thighs	☐	Points of Contact with Clothing (e.g waistband, undergarment)	☐
Lower Legs	☐		☐
Tops of Feet/Toes	☐		☐

.

Appendix 6 Clinical Scratch Scale

VLX-401		
Site #: ____ ____ ____ Date: _____	**Clinician Scratch Scale**	Subject Number: ___ ___ ___

The clinician will rate the subject's pruritus, as evidenced by scratching, according to the following scale:

Score	Description
0	None
1	Rubbing or mild scratching when undistracted
2	Active scratching without evident skin abrasions
3	Abrasion evident
4	Cutaneous mutilation, haemorrhage and scarring evident

Clinician Scratch Scale Score: _____

Clinician Signature: _____

Date: _____

Page **1** of **1**
Protocol: VLX-401
Questionnaire: Clinician Scratch Scale
Version 1.0
28Sep2020

Appendix 7 PROMIS® Fatigue and Sleep Modules

The PROMIS® SF v1.0-Fatigue 7a is the original 7-item short form of the PROMIS® Fatigue measure. It was designed for oncology patients to assess the impact and experience of fatigue from mild subjective feelings of tiredness to sustained sense of exhaustion that decreases one's ability to execute daily activities and function normally in family or social roles. The recall period for the measure is 7 days. Respondents endorse each item using a 5-point response scale: Never, Rarely, Sometimes, Often, and Always. A single item, "How often did you have enough energy to exercise strenuously?," is reverse scored. Developers of the measure provide several ways to score the items, including model-based scores, sum scores, or T scores. The analyses that follow will utilize the T score.

PROMIS Fatigue – Short Form 7a

PROMIS® Item Bank v.1.0 - Fatigue -Short Form 7a

Fatigue - Short Form 7a

Please respond to each question by marking one box per row.

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
FATEXP20	How often did you feel tired?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATEXP5	How often did you experience extreme exhaustion?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATEXP18	How often did you run out of energy?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP33	How often did your fatigue limit you at work (include work at home)?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP30	How often were you too tired to think clearly?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP21	How often were you too tired to take a bath or shower?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP40	How often did you have enough energy to exercise strenuously?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

Source: 31 July 2018 © 2008-2018 PROMIS Health Organization (PHO)

The PROMIS® SF v1.0-Sleep Disturbance in an 8-item measure that assesses quality of sleep, sleep depth, and restoration associated with sleep. All items use a recall period of 7 days. This measure targets no specific sleep disorders and is a general measure of sleep disturbance. All items use a 5-point response scale, the specific response options vary by items. The first 4 items use response options that range from Not at all to Very much. The next 3 items use response options that range from Never to Always. The final item assesses sleep quality ranging from Very poor to Very good. A mix of items are reverse coded. After correcting for reverse coding, model-based scores, sum scores, or T scores are available. These T scores will be used for the analyses.

PROMIS Sleep Disturbance – Short Form 8b

PROMIS Item Bank v1.0 – Sleep Disturbance – Short Form 8b

Sleep Disturbance – Short Form 8b

Please respond to each item by marking one box per row.

	In the past 7 days...	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
Sleep108	My sleep was restless	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep115	I was satisfied with my sleep	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Sleep116	My sleep was refreshing	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Sleep44	I had difficulty falling asleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
	In the past 7 days…	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
Sleep67	I had trouble staying asleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep90	I had trouble sleeping	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep110	I got enough sleep	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
	In the past 7 days…	**Very poor**	**Poor**	**Fair**	**Good**	**Very good**
Sleep109	My sleep quality was	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

Source: 3 May 2016 © 2008-2016 PROMIS Health Organization and PROMIS Cooperative Group

Appendix 8 Patient Impression of Severity-Itch

The Patient Impression of Severity-Itch (PIS-Itch) was designed to assess severity of itching. The PIS-Itch will be completed by participants at time points as specified in the schedule of activities (Table 1). The questionnaire was designed for self-administration and uses a 4-point scale from No itch to Severe. The PIS-Itch scores will be used as anchors to help assess meaningful change for the Adult Itch Reported Outcome (ItchRO) in the study

How would you rate the severity of your worst itch in the past 7 days?

☐ No itch
☐ Mild
☐ Moderate
☐ Severe

Appendix 9 Patient Global Impression of Change-Itch

The Patient Global Impression of Change-Itch (PGIC-Itch) was designed to assess change in itching from baseline after being treated with study drug. The PGIC-Itch will be completed by participants at specified time points in the schedule of activities (Table 1).

The questionnaire was designed for self-administration and uses a 7-point scale from Much better to Much worse. The PGIC-Itch scores will be used as anchors to help assess meaningful change for the Adult Itch Reported Outcome (ItchRO) in the study.

How would you rate the change in your worst itch since the start of the study?

☐ Much better (1)

☐ Better (2)

☐ A little better (3)

☐ No change (4)

☐ A little worse (5)

☐ Worse (6)

☐ Much worse (7)

Appendix 10 List of Known OATP2B1 Substrates

The following drugs are known to be OATP2B1 substrates:

- Bosentan
- Bromsulphthalein
- Dehydroepiandrosterone
- Estrone-3-sulfate
- Fexofenadine
- Glyburide
- Quercetin
- Scutellarin (herbal)
- Statins
    - Atorvastatin
    - Fluvastatin
    - Pitavastatin
    - Pravastatin
    - Rosuvastatin
- Sulfasalazine
- Telmisartan
- SN-38 carboxylate (topoisomerase inhibitor metabolite)
- SN-38 lactone (topoisomerase inhibitor metabolite)

Appendix 11 Management of Clinical Study Procedures and Participants during COVID-19 Pandemic or Other Force Majeure

This appendix provides guidance for participant safety and ongoing access to medical care and investigational product during the global novel coronavirus 2019-nCoV (COVID-19) pandemic. During this global public health crisis, pragmatic and harmonized actions are required to ensure the necessary flexibility and procedural simplifications that are needed to maintain the integrity of the clinical studies and to ensure the rights, safety, and wellbeing of study participants and the safety of clinical study staff.

The measures detailed here are implemented across Mirum studies on a temporary basis until the pandemic is considered resolved by governmental and public health organizations, as applicable.

Regardless of the guidance provided below, consideration should be given to public health advice at the study locations and individual benefit/risk in treatment decisions for participants at the study site during the pandemic (EMA 2020; FDA 2020). Logistical requirements should also be considered, such as the ability of participants to travel to the study site, accessibility of public transportation, site restrictions on clinical studies, etc.

The safety of the study participants is of primary importance, and risks of involvement in the study, in particular with added challenges due to COVID-19, will be weighed against anticipated benefit for the study participants and society.

Study Participation

Study sites may continue to recruit new participants, if deemed appropriate on benefit-risk assessment as described and provided that ALL of the following activities to preserve study integrity can be met:

- Upon discussion with the site monitor, the study site has confirmed the ability to enroll and manage studies and participants effectively and in compliance with the protocol and this appendix.
- Upon discussion with the sponsor or designee, the study site has confirmed that appropriate safety monitoring can take place, in compliance with the protocol and this appendix.
- Data will continue to be entered into the electronic case report form (eCRF) and queries resolved in a timely manner.
- The site monitor is able to access the study site to perform onsite monitoring or is able to perform remote monitoring of data.

Conduct of Telephone/Virtual or Alternative Visits

Due to the current pandemic, it is conceivable that not all participants visit commitments can be fulfilled. If a participant or caregiver is unable or unwilling to attend a study visit or a study site is unable to permit onsite visits by participants and caregivers, adaptation of the onsite visit to a telephone visit, virtual visit, or use of an alternative location for assessment (including but not limited to local laboratories, family physician, home visit) is recommended to ensure continuity of participant care during the study (as an interim measure). Priority should be given to

maintaining ongoing safety follow-up. Study sites should speak with their site monitor before performing a telephone or virtual visit so he or she may provide guidance regarding logistics that may need consideration. Study sites should contact the medical monitor if the participant must miss >1 onsite visit in succession, because multiple incomplete visits may have the potential to impact evaluation of study endpoints as well the participant's safety.

The decision to accept protocol deviations for missed assessments is based on the risk to the participant of discontinuing study participation versus missed assessments, such as safety laboratory assessments. Where possible, a home care visit should be organized to avoid missing visits or assessments.

Alternative Blood Sample Collection

Where possible and permitted, homecare can be organized through qualified health care professionals, trained on the study protocol, to collect samples at the participant's home (or other agreed upon location). It is preferred that this alternative blood sample collection is performed in a manner to support delivery of samples to the central laboratory, but the analysis of samples may be performed at local laboratories, if necessary. If the analyses are performed at the local laboratories, the site will follow up with the participant to obtain the results and local laboratory reference ranges for data entry into EDC as soon as they are available.

Safety Reporting and Volixibat Treatment Recommendations for COVID-19

The sponsor recognizes that COVID-19 presents an increased risk for all participants. Due to the potential impact of COVID-19 on multiple organ systems, the sponsor recommends the following dose modification and management plan for participants with confirmed or suspected COVID-19 while receiving treatment with volixibat.

The following safety reporting guidelines are required:

- All confirmed or suspected COVID-19–related adverse events (AEs) must be recorded in the eCRF and the Serious Adverse Event (SAE) Report Form if the event meets the seriousness criteria. SAEs are reportable to the sponsor immediately without undue delay after becoming aware of the event and no later than 24 hours of awareness. All study-drug interruptions or modifications must be recorded on the AE and drug administration eCRFs.
- If an event is suspected to be COVID-19 infection, continuation of study drug, and any dose modifications should be assessed on a case-by-case basis, depending on the participant's health status, and discussed with the medical monitor if needed. The COVID-19 infection should be managed as per local treatment guidance and investigator's clinical judgment.

Drug-Drug Interaction Guidance Regarding Medication Used to Treat COVID-19

- The study protocol and Investigator Brochure should be referenced for drug-drug interaction information. For further information the medical monitor can be contacted.

Guidance Regarding Vaccination against COVID-19

- Participants should be requested to contact the site upon planned vaccination against COVID-19. The vaccine should be entered in the eCRF as a concomitant medication and any new or worsened symptom or sign recorded as an adverse event and assessed for seriousness. Volixibat is taken orally, is minimally absorbed with systemic levels below the lower limit of quantification, and binds with and inhibits the ileal bile acid transporter (IBAT) in the distal ileum. The vaccine is administered and acts systemically; hence, no drug-drug interaction is expected, and no need for unblinding is foreseen.

Documenting Alternative Contacts with Participants

If an onsite visit is replaced by a telephone or virtual study visit, the following are guidelines for data capture:

- If the visit is listed as onsite but is performed remotely, data should be captured as per a normal visit (i.e., document the remote visit in the source documents and complete the relevant eCRF pages to capture the visit date, and any possible assessment that can be obtained, such as AEs, study drug administration, and/or concomitant medications and any additional safety or efficacy information). All assessments that cannot be performed should be marked as not done in the eCRF. Any protocol deviations related to COVID-19 should be recorded in the clinic notes, prefixed COVID-19.
- Visits that require procedures that cannot be performed remotely should be discussed with the site monitor because this may impact efficacy or safety analyses and should be documented as a protocol deviation due to COVID-19.

Site-to-Participant Drug Shipment Instructions during Pandemic Containment

If a participant is unable to go to the study site because of pandemic containment, the study site may ship the study drug to the home of the participant (or other agreed location) following approval by the sponsor.

For such shipment, the following conditions must be met:

- The sponsor is responsible for delivery of the study drug to the study site. Organization of shipments from the site to the participant will be the responsibility of the study site.
- The participant or caregiver is informed about the shipment method, confirms the address for receipt of the drug, and agrees that his or her personal information (i.e., contact information) may be given to a professional carrier.
- The investigator or designee (e.g., pharmacy) securely packages the drug for shipment.
- A professional carrier is used by the investigator or designee to ship the drug securely and maintain chain of custody, with evidence provided. Volixibat should be stored

and shipped as directed in the Pharmacy Manual. Shipments that are expected to be completed within 48 hours from pickup to delivery do not generally require any specific temperature monitoring.

- To respect participant confidentiality, the carrier should be given only the contact information of the recipient. The sponsor should not receive any personal information about the participant.
- A procedure is defined with the carrier to immediately upon delivery confirm the receipt of the drug by the recipient and that it is received in good condition.
- The site contacts the recipient to confirm the receipt of the drug and to confirm it is delivered in the expected conditions (e.g., not leaking, not broken) and gives instructions about the drug administration and storage. If the drug is delivered and is not within the expected conditions, the site will give instructions to the participant about the next steps.
- The investigator or designee completes the drug accountability forms with each shipment made directly to a participant.
- All documentation (originals/copies, as applicable) related to the site-to-participant drug shipment should be filed in the investigator site file, including the list of the medication being delivered, the quantity involved, and documentation of receipt by the study participant.
- Participants should retain unused study drug and containers and return them to the investigator the next time they visit the investigator site

COVID-19 Specific Remote Source Data Verification

The source documents/source data considered for remote source data verification (rSDV) are those related to the primary endpoint, AEs/SAEs, important medical events, or the reasons for exclusion of a participant from the study.

Remote access to source documents/source data for monitoring purposes may only take place in justified exceptional cases and only to the extent strictly necessary (i.e., only when direct access to study source data is not available due to COVID-19 pandemic restrictions).

The following 3 options for source data reconciliation without the study monitor being physically present at the study site may be used, based on the site-specific source documentation/source data:

- The study site will provide the study monitor, under the responsibility of the investigator, with copies of the source documents/source data in which personal identifying information of the study participants and information pertaining to their privacy has been obscured or redacted.
- Under the responsibility of the investigator, the study site will grant the study monitor direct, controlled, remote access to the systems with which the source documents/source data are managed.

- The study site will grant the study monitor, under the responsibility of the investigator, passive access to the source documents/source data via live image transmission (e.g., sharing of the screen or image/sound transmission).

Depending on the option selected for rSDV, the full process to be followed and requirements needed to be met are described in the specific rSDV section of the clinical monitoring plan as well as in a corresponding guideline for the investigators.

In the case of live image transmission, this will take place exclusively via secured systems/environments and systems/servers within European Economic Area/European Union.

In all cases, rSDV will be conducted exclusively by the authorized persons in accordance with the written informed consent of the study participants and the written agreement of the Principal Investigator (PI)/PI's institution.

Remote source data verification will take place in a protected environment (i.e., providing protection from unauthorized access in any form, including the use of privacy screens to prevent unauthorized viewing of source documents/source data), the source documents/source data reviewed by the study monitor will not be permanently stored by the study monitor, and if necessary, temporarily saved files (including ones automatically generated by the system) are permanently deleted at short notice.

The study monitor will securely destroy any copy of obscured/redacted documents, whether paper or electronic, as soon as they have been used for source data verification.

Appropriate corrective measures will be implemented in the event of technical difficulties or if the security of the transmission is no longer guaranteed.

The information and communication technology used by the sponsor and the study site for rSDV is designed in such a way that secure and General Data Protection Regulation–compliant transmission is guaranteed.

REFERENCES

United States Food and Drug Administration. FDA guidance on conduct of clinical trials of medical products during COVID-19 public health emergency. March 2020 (updated 30 August 2021). Available online at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/fda-guidance-conduct-clinical-trials-medical-products-during-covid-19-public-health-emergency.

European Medicines Agency. Guidance on the management of clinical trials during the COVID-19 (coronavirus) pandemic. Version 3, 28 April 2020 (updated Version 4, 4 February 2021). Available online at https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/guidanceclinicaltrials_covid19_en.pdf.