A PHASE 3 STUDY TO EVALUATE THE EFFECTS OF CHENODEOXYCHOLIC ACID IN ADULT AND PEDIATRIC PATIENTS WITH CEREBROTENDINOUS XANTHOMATOSIS (RESTORE)

Investigational Medicinal Product:		Chenodeoxycholic Acid (CDCA)
Protocol Number:		Cheno-CTX-301
IND Number:		IND 124960
NCT Number:		NCT04270682
Developmental Phase:		Phase 3
	Amendment 6	04 April 2023
	Amendment 5	23 August 2021
	Amendment 4	15 April 2020
	Amendment 3	22 July 2019
	Amendment 2	05 April 2019
	Amendment 1	19 December 2018
	Original Protocol	13 November 2018
Sponsor:		Travere Therapeutics, Inc. 3611 Valley Centre Drive, Suite 300 San Diego, CA 92130 USA +1 888-969-7879

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INVESTIGATOR'S AGREEMENT

This protocol was designed and will be conducted, recorded, and reported in accordance with the principles of GCP as stated in the ICH of Technical Requirements for Registration of Pharmaceuticals for Human Use and any applicable national and regional laws.

I have read and agree to abide by the requirements of this protocol.

Investigator's Signature	Date

Investigator's Name:

Institution Name:

Institution Address:

Institution Phone Number:

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1. SYNOPSIS

Name of Sponsor/Company: Travere Therapeutics, Inc.		
Name of Investigational Product: Chenodeoxycholic Acid (CDCA)		
Name of Active Ingredient: chenodeoxycholic acid; 3α, 7α-dihydroxy-5β-cholan-24-oic acid		
Protocol No.: Cheno-CTX-301	**Phase:** 3	**Country:** Global
Title of Study: A Phase 3 Study to Evaluate the Effects of Chenodeoxycholic Acid in Adult and Pediatric Patients with Cerebrotendinous Xanthomatosis (RESTORE)		
Study Center(s): Approximately 10 investigational study centers globally will participate in this study.		
Studied Period (years): Estimated date first patient enrolled: November 2019 Estimated date last patient completed: December 2023		**Phase of Development:** Phase 3

Objectives:

Efficacy Objective:

- Determine the effects of chenodeoxycholic acid (CDCA), compared with placebo, on biomarkers and clinical symptoms of cerebrotendinous xanthomatosis (CTX) in adult patients with CTX.

Safety Objective:

- Assess the safety and tolerability of CDCA in adult and pediatric patients.

Exploratory Objectives:

- Characterize the relationship among biomarkers in adult and pediatric patients.
- Characterize steady-state pharmacokinetics (PK) of CDCA and 2 conjugated forms of CDCA, GlycoChenodeoxycholic Acid (gCDCA) and TauroChenodeoxycholic Acid (tCDCA) in adult patients.
- Characterize the relationship between dose and PK concentration among pediatric patients.
- Assess biomarkers and health status among pediatric patients treated with CDCA.

Methodology:

This study is made up of 2 designs: a randomized, double-blind (DB), crossover study design among patients ≥16 years of age at Screening (adult cohort) and an open-label (OL), dose-titration, and maintenance study design among pediatric patients ≥1 month and <16 years of age at Screening (pediatric cohort).

ADULT COHORT:

Patients in the adult cohort will participate in a randomized, double-blind, placebo-controlled, 2-period × 2-treatment crossover study with rescue medication to assess the efficacy and safety of CDCA in the treatment of CTX. Approximately 12 male and/or female CDCA-naïve or CDCA-treated patients ≥16 years of age at Screening with CTX meeting all inclusion criteria and none of the exclusion criteria and whose clinical symptoms are stable during the OL run-in period (OL1) are planned to be randomized in the study. At least 6 of the patients randomized in the study will be treatment-experienced (ie, those who have been on a stable dose of CDCA for at least 2 months prior to the run-in period). Patients who are either CDCA-naïve or those with less than 2 months of treatment prior to the run-in period are considered newly initiated patients.

Patients will be screened at Visit 1 (Screening; Appendix 1) and those who satisfy all the inclusion and none of the exclusion criteria will participate in the 8-week OL1 to establish reliable baseline measurements for biomarkers and other efficacy assessments. Patients who fail screening may be rescreened as needed.

Adult cohort patients (≥16 years of age) will receive 250 mg open-label CDCA 3 times daily (TID) during the OL periods of the study (ie, OL1 and OL2). During the DB periods (DB1 and DB2), adult cohort patients will receive either blinded CDCA 250 mg TID or matching placebo TID.

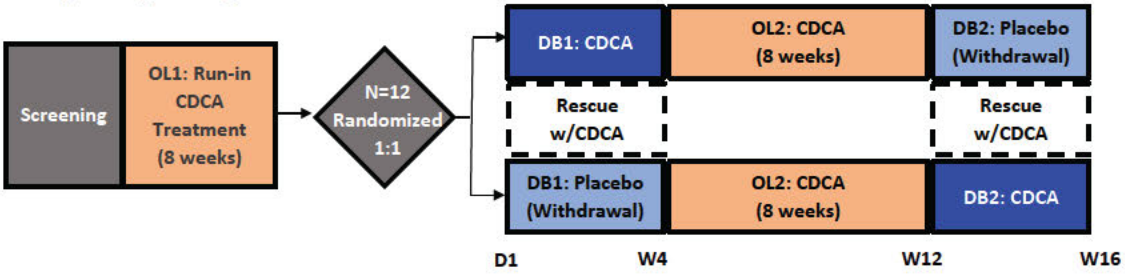
Following the OL1 period, patients will be reassessed for eligibility before randomization (Day 1, Visit 6 [Appendix 1]). To be randomized, patients are required to have tolerated CDCA and not have developed any condition that, in the judgment of the Investigator, warranted discontinuation of CDCA treatment.

Adult cohort patients will be randomized on Day 1 at a 1:1 ratio to 1 of 2 treatment sequences (AB or BA) to enter a 4-week DB period (DB1), an 8-week OL period (OL2), and then a second 4-week DB period (DB2).

Visits at an alternative site by a trained medical professional (eg, licensed nurse) will be permitted to reduce the travel burden for patients. The visits that qualify for being conducted at an alternative site by a home health vendor are indicated in the Adult Cohort Schedule of Assessments (Appendix 1).

After the last dose of study medication, patients may transition to treatment for CTX per the guidance of the Investigator or prescribing physician. A safety follow-up phone call will be conducted for all patients 30 (±7) days after the last dose of study medication.

Study Design Diagram for Adult Cohort:



CDCA = chenodeoxycholic acid; D = day; DB1 = double-blind period 1; DB2 = double-blind period 2; OL1 = open-label period 1; OL2 = open-label period 2; W = Week.

Note 1: Patients will be contacted (via telephone call) 30 (±7) days after the last dose of study medication to ascertain patient safety.

Note 2: Patients randomized to placebo will receive blinded CDCA rescue if biochemical criteria are triggered and open-label CDCA if new or worsening CTX-related symptoms trigger rescue during DB1 or DB2. Patients randomized to CDCA will continue to receive blinded CDCA rescue if triggered by biochemical criteria and will receive open-label CDCA rescue if new or worsening CTX-related symptoms are present.

4-Week DB1: On Day 1, patients will be randomized to treatment sequence AB or BA. Patients randomized to sequence AB will receive blinded 250 mg CDCA TID for 4 weeks or until rescue medication criteria are triggered; patients randomized to sequence BA will receive placebo TID for 4 weeks or until rescue medication criteria are triggered. Should rescue medication criteria be met, patients will receive 250 mg CDCA TID until the end of DB1. Patients will return to the clinic on Day 8 and weekly thereafter for the remaining 3 weeks.

8-Week OL2: Upon completion of DB1, patients will receive open-label 250 mg CDCA TID for 8 weeks and return to the clinic for biweekly scheduled visits.

4-Week DB2: At Week 12, patients who were randomized to sequence AB will crossover into DB2 and receive placebo treatment TID for 4 weeks or until rescue medication criteria are triggered; patients who were randomized to sequence BA will receive blinded 250 mg CDCA TID treatment for 4 weeks or until the rescue medication criteria are triggered. Should rescue medication criteria be met, patients will receive 250 mg CDCA TID until the end of DB2. Patients will return to the clinic on Day 92 (7 days after the first day of the DB2 period), and weekly thereafter for the remaining 3 weeks.

Rescue Medication Criteria in the Adult Cohort:

Blinded or open-label 250 mg CDCA TID rescue medication will be provided during the DB periods, if needed, based on blinded biomarkers (blinded rescue) and/or clinical CTX-related symptoms (open-label rescue). Patients who require blinded and/or open-label CDCA rescue medication in a given period will remain on the rescue medication for the remainder of the respective DB period. Patients who require rescue medication during DB1 but otherwise remain eligible should continue to OL2 and then to DB2.

Blinded CDCA will be provided to patients who meet the following criterion: increase of 10 times the baseline value for a given period (ie, Visits 6 and 14) in urine 23S-pentol. Blinded CDCA will be assigned through the Interactive Voice/Web Response System (IxRS) at the next scheduled in-clinic visit. The Investigator and patient will remain blinded to biomarker data, treatment assignment, and if blinded rescue medication criteria are met.

Patients receiving either blinded CDCA or blinded placebo during the DB period will be rescued with open-label CDCA at the discretion of the Investigator if they present with new or worsening CTX-related symptoms relative to their respective pretreatment assessments as defined in the protocol. The Investigator is responsible for evaluating patients to determine if they meet the criteria for rescue medication at each follow-up visit during DB1 and DB2 (Appendix 1).

Extension of an Open-Label Period as a Result of COVID-19:

An adult cohort patient's participation in an OL period (ie, OL1 or OL2) may be extended to delay the start of a DB withdrawal period (ie, DB1 or DB2) if study and/or site operations are disrupted as a result of the Coronavirus Disease 2019 (COVID-19) pandemic. The decision to postpone the start of a DB period will be at the discretion of both the Investigator and Medical Monitor. If it is decided to extend an OL period, the patient will return to the study site once a month from the Visit 4 (OL1) or Visit 12 (OL2) date or last dispensation of OL study medication during the respective OL period. During these extension visits, the Investigator will ensure patient safety monitoring and dispensation of additional OL study medication is performed. Efficacy assessments during the extension visits are optional. See Appendix 6 for the Schedule of Assessments.

Patients will continue to receive 250 mg open-label CDCA TID during the extension periods. Details within the clinical study protocol regarding study medication in the OL1 and OL2 periods are applicable for the extension periods. A patient's total duration on treatment may extend beyond 24 weeks if an extension visit is required because of COVID-19.

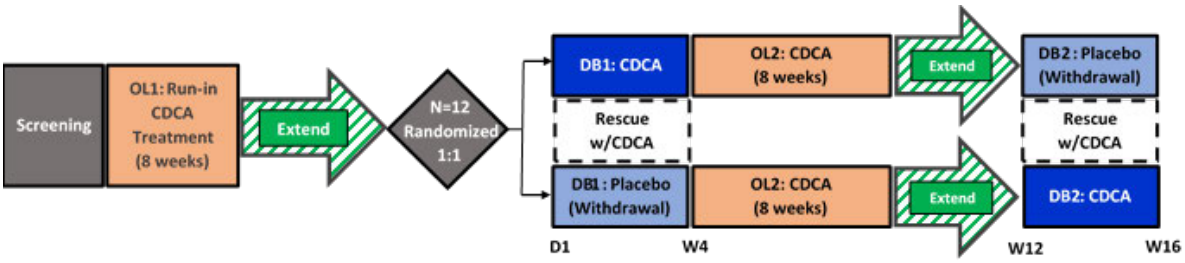
A patient may proceed with the start of a DB withdrawal period when the Investigator and Medical Monitor agree that the impact to site and/or study operations because of COVID-19 has stabilized. The Investigator and Medical Monitor will consider the site's ability to perform study visits without

interruption and the timely reporting of necessary study results. A patient will resume the patient schedule as detailed in Appendix 1 when starting a DB period.

If a patient completes 2 or more extension visits in 1 OL period, the patient must complete a study Visit 2 weeks prior to the start of a DB period to complete all safety and efficacy assessments that were otherwise required during a visit in the OL period.

Visits at an alternative site by a trained medical professional (eg, licensed nurse) will be permitted to reduce the travel burden for patients. The visits that qualify for being conducted at an alternative site by a home health vendor are indicated in the Schedule of Assessments (Appendix 6).

Study Design Diagram for Extension of Open-Label Period in the Adult Cohort:



CDCA = chenodeoxycholic acid; D = day; DB1 = double-blind period 1; DB2 = double-blind period 2; OL1 = open-label period 1; OL2 = open-label period 2; W = Week.

PEDIATRIC COHORT:

Pediatric cohort patients (≥1 month and <16 years at Screening) will participate in a 24-week, open-label cohort with an 8-week titration period to identify a safe and tolerable dose and a 16-week treatment period at the safe and tolerated dose as maintenance. Patients will be screened at Visit 1 (Appendix 2) and those who satisfy all the inclusion criteria and none of the exclusion criteria will be eligible to participate in this cohort to evaluate safety, PK, and biomarkers in this population. Patients will have visits every week during the titration period and every 4 weeks during the treatment period.

During the dose-titration period, dose escalation decisions will be based on safety and tolerability (Section 6.5.1).

Dosing will be as follows:

- Treatment-naïve pediatric cohort patients will receive doses of 5 mg/kg/day (TID), 10 mg/kg/day (TID), or 15 mg/kg/day (TID) titrated up every 2 weeks based on safety and tolerability over a period of 8 weeks.
- Treatment-experienced pediatric cohort patients currently taking a dose ≥5 mg/kg/day of CDCA will start at their current dose and titrate up every 2 weeks to the next dose level up to 15 mg/kg/day based on safety and tolerability over a period of 8 weeks. Patients with a history demonstrating intolerance at higher doses will not be required to dose escalate.
- Pediatric dosing of CDCA will not exceed an equivalent dose of 750 mg/day.
- Pediatric cohort patients who are able to take an equivalent weight-based dose of 750 mg/day and are capable of swallowing a tablet will have the option to dose with CDCA 250 mg tablets TID or the liquid suspension form of CDCA.

During the treatment period, dosing will be maintained at the dose tolerated during the titration period.

After the last dose of study medication, patients may transition to treatment for CTX per the guidance of the Investigator or prescribing physician. A safety follow-up phone call will be conducted for all patients 30 (±7) days after the last dose of study medication.

Study Design Diagram for Pediatric Cohort:

D = day; W = Week.

Unblinded Data Monitoring Committee:

An unblinded Data Monitoring Committee (DMC), including physicians with experience treating patients with CTX, physicians trained in neurology, internal medicine, and/or pharmacovigilance, as well as statistician(s) will monitor accumulating data, including biomarkers (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4). The DMC will review efficacy, clinical outcomes, and safety results to potentially stop the study early based on the overall benefit-risk assessment, including the risk of irreversible disease progression among those receiving placebo.

Number of Patients (Planned):

Approximately 12 patients ≥16 years of age at Screening are planned to be randomized in the adult cohort.

Pediatric patients ≥1 month and <16 years at Screening will be enrolled separately in the pediatric cohort.

Diagnosis and Main Criteria for Inclusion:

Inclusion/Exclusion Criteria:

Eligibility must be confirmed and signed/dated informed consent must be obtained prior to any study-related procedure from the patient or from the parent/legal guardian if the patient is <18 years or mentally incapacitated. Assent must be obtained prior to any study-related procedure from patients who are <18 years old or patients who are mentally incapacitated if, in the Investigator's opinion, the patient is able to understand and provide assent.

Inclusion Criteria:

Male and female patients with a clinical diagnosis of CTX are eligible for this study if they meet all of the following criteria:

1. The patient or parent/legal guardian (as appropriate) is willing and able to provide signed informed consent, and where required, the patient is willing to provide assent, prior to any screening procedures.
2. The patient is ≥1 month of age at the time of signing the informed consent and assent, as applicable.
3. Clinical diagnosis of CTX with biochemical confirmation. For treatment-experienced patients, documented historical confirmation of serum cholestanol and/or bile alcohol is acceptable. For

treatment-naïve patients, serum cholestanol and urine 23S-pentol levels will be obtained prior to initiating open-label CDCA.

4. If the patient is currently being treated with CDCA at a dose different than the protocol-specified dose, the patient must be willing to change his or her current dose.
5. With Medical Monitor approval, patients currently taking CDCA and riluzole for the treatment of ataxia are eligible, provided their ataxia is well controlled and they have been on a stable dose of riluzole for a minimum of 6 months.
6. Women of childbearing potential (WOCBP), beginning at menarche, must agree to the use of 1 highly reliable (ie, can achieve a failure rate of <1% per year) method of contraception during the course of the study. Highly reliable contraception methods include stable oral, implanted, transdermal, or injected contraceptive hormones associated with inhibition of ovulation, or an intrauterine device (IUD) in place for at least 3 months. One additional barrier method must also be used during sexual activity, such as a diaphragm with spermicide or male partner's use of male condom with spermicide, during the course of the study. WOCBP are defined as those who are fertile, following menarche and until becoming postmenopausal unless permanently sterile; permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A postmenopausal state is defined as amenorrhea for more than 24 consecutive months without an alternative medical cause; women on hormone replacement therapy must have a documented plasma follicle-stimulating hormone level >40 mIU/mL. All WOCBP must have a negative serum pregnancy test at Screening. NOTE: Prior to menarche, pregnancy testing and contraceptive use is not required. However, the patient and their parent/legal guardian must be advised that, immediately upon menarche, the patient will be required to begin pregnancy testing and initiate contraceptive use. This requirement cannot be avoided.
7. Males must be surgically sterile (more than 3 months post-vasectomy) or males and their sexual partners must together agree to the use of medically accepted methods of contraception that are considered highly reliable during the course of the study.
8. The patient and/or his/her parent/legal guardian is willing and able to comply with the protocol.

Exclusion Criteria:

A patient who meets any of the following criteria will be excluded from this study:

1. The patient has a negative genetic sequencing result for CTX.
2. The patient is known to have another malabsorption disorder or confounding inflammatory gastrointestinal condition (eg, irritable bowel syndrome).
3. The patient has been diagnosed with New York Heart Association (NYHA) Class II – IV heart failure.
4. If the patient is receiving anticoagulants and, in the judgment of the Investigator, is not well-controlled.
5. The patient is taking medications which impact bile acid absorption such as bile acid sequestering agents (eg, cholestyramine, colestipol, aluminum-based antacids).
6. The patient is taking cholic acid medication.

7. The patient is pregnant or lactating or presents with a positive serum pregnancy test at Screening or a positive urine pregnancy test at the first day of OL1 for adult cohort or Titration Start for pediatric cohort.
8. The patient has been (or is currently) enrolled in a clinical study involving study medication or device within 30 days prior to Screening.
9. The patient has other prior or ongoing medical conditions, physical findings, or laboratory abnormalities that, in the Investigator's opinion, could adversely affect the safety of the patient, make it unlikely that the course of treatment or follow up would be completed, or impair the assessment of study results.
10. The patient has a history of drug or alcohol abuse within the past year that, in the judgment of the Investigator, could affect participation in or adherence to the requirements of the study.
11. The patient has a positive serologic test for hepatitis B virus surface antigen, hepatitis C, or human immunodeficiency virus at Screening.
12. The patient and/or his/her parent/legal guardian, in the opinion of the Investigator, is unable to adhere to the requirements of the study.

Inclusion Criteria Pre-Randomization for the Adult Cohort:

Patients in the adult cohort who meet all inclusion criteria and none of the exclusion criteria for participation into the run-in period **AND**

1. Tolerate CDCA treatment, in the judgment of the Investigator.
2. Have not developed any condition that, in the judgment of the Investigator, warrants discontinuation of CDCA.

Patients who fail screening may be rescreened as needed.

Study Medication, Dosage and Mode of Administration:

Adult cohort patients (≥16 years): CDCA tablets, 250 mg TID, oral

Pediatric cohort patients (≥1 month and <16 years): CDCA tablets will be carefully crushed by the pharmacist, added to a sodium bicarbonate solution 8.4% (1 mmol/mL) to create a 10 mg/mL liquid suspension (ie, one 250 mg tablet per 25 mL) and supplied for oral administration. Patients who are able to take an equivalent weight-based dose of 750 mg/day and are capable of swallowing a tablet will have the option to take CDCA 250 mg tablets TID or the liquid suspension form of CDCA.

Doses will be 5 mg/kg/day (TID), 10 mg/kg/day (TID), and 15 mg/kg/day (TID) depending upon the safe and tolerated titrated dose.

- Treatment-naïve pediatric cohort patients will receive doses of 5 mg/kg/day (TID), 10 mg/kg/day (TID), or 15 mg/kg/day (TID) titrated up every 2 weeks based on safety and tolerability over a period of 8 weeks.
- Treatment-experienced pediatric cohort patients currently taking a dose ≥5 mg/kg/day of CDCA will start at their current dose and titrate up every 2 weeks to the next dose level up to 15 mg/kg/day based on safety and tolerability over a period of 8 weeks. Patients with a history demonstrating intolerance at higher doses will not be required to dose escalate. Pediatric dosing of CDCA will not exceed an equivalent dose of 750 mg/day.

Patients currently taking Chenodal® and/or an alternative therapy for CTX are required to stop their medication/therapy prior to the start of study medication in OL1 for the adult cohort or the titration

period for the pediatric cohort. Patients may resume or start taking Chenodal® and/or an alternative therapy for CTX after completing the study (ie, EOS visit) or permanently discontinuing study medication.

Duration of Study:

The adult cohort will be approximately 24 to 28 weeks in duration, including an up to 4-week screening period; an 8-week, open-label run-in period (OL1); two 4-week, DB treatment periods (DB1 and DB2); and an 8-week open-label treatment period (OL2) in between DB1 and DB2. The duration of the adult cohort may extend beyond 28 weeks if the start of a double-blind period is delayed due to COVID-19.

The pediatric cohort will be approximately 24 to 28 weeks in duration, including an up to 4-week screening period; an 8-week, open-label dose-titration period; and a 16-week, open-label treatment period at the identified safe and tolerated dose as maintenance.

If genetic results are still pending after 28 days, the screening window for both cohorts may be extended up to 2 additional weeks, with approval by the Medical Monitor, which will bring the maximum total duration of the study to 30 weeks.

For both cohorts, a safety follow-up phone call will be conducted 30 (±7) days after the last dose of study medication.

Reference Therapy, Dosage and Mode of Administration:

CDCA will be compared with placebo in the adult cohort. There is no reference therapy.

Criteria for Evaluation:

Efficacy: Urine bile alcohols (23S-pentol), plasma bile alcohols (tetrol glucuronide), plasma cholestanol, plasma7αC4, and plasma 7α12αC4.

Rescue or Disease Progression: Patient diary and health status questionnaire, clinician global impression of change, and developmental milestones (pediatric cohort only).

Safety: Evaluation of treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), vital signs, safety laboratory tests (hematology, chemistry, coagulation [adult cohort only], lipid profile [including cholesterol], and urinalysis), 12-lead electrocardiogram (ECG), electroencephalogram (EEG), and physical examination (including body weight).

Primary Efficacy Endpoint for the Adult Cohort:

- Change from baseline in $\log_e$-transformed urine 23S-pentol at the end of each DB treatment period

Key Secondary Efficacy Endpoints for the Adult Cohort:

- Proportion of patients requiring rescue medication during the DB periods
- Percent change from baseline in plasma cholestanol levels at the end of each DB treatment period
- Percent change from baseline in plasma 7αC4 at the end of each DB treatment period

Other Secondary Efficacy Endpoints for the Adult Cohort:

- Change from baseline in plasma cholestanol to cholesterol ratio during the DB periods
- Percent change from baseline in plasma 7α12αC4 at the end of each DB treatment period
- Proportion of patients with negative net change in symptoms and manifestations reported in the diary during the DB periods

Safety Endpoints for both Adult and Pediatric Cohorts:

- Change from baseline in body weight, vital signs, physical examinations, EEG, 12-lead ECG, clinical laboratory parameters (hematology, chemistry, coagulation [adult cohort only], lipid profile [including cholesterol]), and urinalysis laboratory parameters at each visit where parameters are collected
- Incidence of TEAEs, including SAEs, adverse events (AEs) leading to discontinuation of study medication, and AEs of interest (AEOI)

Exploratory Endpoints for Both Adult and Pediatric Cohorts:

Adult cohort patients:

- Percent change from baseline in plasma bile alcohol at the end of each DB treatment period
- Steady-state PK parameters of 3 bile acids that include CDCA, gCDCA, and tCDCA and total CDCA (summed CDCA, gCDCA, and tCDCA). (AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, $t_{max,ss}$, CL/F)
- Relationships among biomarkers (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4)
- Relationships between PK exposure parameters (AUC_{ss}, $C_{max,ss}$ or $C_{min,ss}$) and changes in biomarker levels (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4 and plasma 7α12αC4)
- Relationships between PK exposure parameters (AUC_{ss}, $C_{max,ss,}$ or $C_{min,ss}$) and safety endpoints
- Change in disease severity as assessed by the CGI-C during each DB treatment period
- Change in health status questionnaire

Pediatric cohort patients:

- Relationships among biomarkers (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4)
- Steady-state PK concentration of CDCA, gCDCA, and tCDCA and total CDCA (summed CDCA, gCDCA, and tCDCA)
- Mean biomarker levels (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4)
- Change in disease severity as assessed by the CGI-C during the treatment period
- Change in health status questionnaire
- Change in developmental milestones

Statistical Methods:

Descriptive statistics will be produced separately for adult and pediatric cohorts. The following sections describe inferential statistical testing procedures for efficacy endpoints collected during the double-blind period among adult cohort patients.

Analysis of Efficacy Endpoints:

The primary analysis of the primary endpoint of change from baseline in $\log_e$-transformed urine 23S-pentol at the end of each DB treatment period will be conducted via a paired t-test comparing CDCA with placebo using the Full Analysis Set (FAS; adult cohort). Log-transformation is performed due to the anticipated wide range of urine 23S-pentol levels among CTX patients. Urine 23S-pentol will be determined using first morning void urine samples and will be calculated as the geometric mean of 3 first morning void urine samples collected within 5 days prior to each visit at which urine 23S-pentol is assessed. The baseline value for the first double-blind period is the measurement taken

for Day 1; the baseline value for the second double-blind period is the measurement taken at Day 85 at the beginning of the second double-blind period. Measurements obtained after initiation of rescue medication will be considered "missing" for purposes of the primary analysis. An appropriate multiple imputation method will be used to impute missing values and the values after initiating rescue medication, under the assumption of missing at random. The imputed values, not the actual values obtained after the initiation of the rescue medication, will be used in the analysis. Further information on imputation of missing data methods and sensitivity analyses will be described in the Statistical Analysis Plan (SAP). Percent change from baseline in plasma cholestanol levels, and plasma 7αC4 and 7α12αC4 levels at the end of each DB treatment period will be analyzed in a similar way as for the primary endpoint.

The proportion of patients requiring rescue treatment and the corresponding exact 95% confidence interval (CI) will be presented for each treatment. Patients who require blinded and/or open-label CDCA during the DB periods will be considered to have met the event (ie, received rescue medication) for the primary analysis. The difference in proportions, the corresponding exact CI, and exact p-values will be calculated and presented using Prescott's method (Prescott 1981). This analysis will be performed on the FAS.

Other continuous efficacy endpoints will be analyzed using the same approach described for the primary endpoint. Other binary efficacy endpoints (such as incidence of new or worsening in CTX-related clinical manifestations) will be analyzed using the same approach described for the proportion of patients requiring rescue treatment.

Control of Type 1 Error

The primary endpoint of urine 23S-pentol will be tested at the final analysis at an $\alpha = 0.05$. If the test is significant, the Family One is rejected and the entire $\alpha = 0.05$ is preserved and then passed onto Family Two of hypotheses (ie, key secondary endpoints) for testing at the final analysis. Otherwise, there is no remaining α to be passed onto Family Two, and therefore, the key secondary endpoints will not be formally tested, and nominal p-values will be reported instead.

Analysis of Safety Endpoints:

Descriptive statistics will be used to summarize the safety data by randomized treatment group among adult cohort patients based on the safety analysis set (SAS) and by administered dose among pediatric cohort patients based on the pediatric safety analysis set (PSAS).

Clinical laboratory parameters will be measured at baseline and postbaseline visits. Each continuous laboratory variable will be summarized in terms of changes from baseline by treatment group. Laboratory data will also be classified as low, normal, or high relative to the parameter's reference range. Laboratory abnormalities for each treatment will also be summarized using shift tables.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary by System Organ Class and Preferred Term. AEs that begin after the first administration of study medication, or existing AEs that worsen after the first dose of study medication, are considered TEAEs. The number and percentage of patients reporting TEAEs will be summarized for each treatment group by MedDRA System Organ Class and Preferred Term, by severity, and by relationship to study medication. The number and percentage of patients reporting serious TEAEs, TEAEs leading to treatment discontinuation, and AEOIs will also be summarized for each treatment group by MedDRA System Organ Class and Preferred Term.

Sample Size Justification for Adult Cohort:

The hypothesis to be tested is H_0: $\Delta = 0$ against H_a: $\Delta \neq 0$, where Δ is the true paired treatment difference between CDCA and placebo for the change from baseline in $\log_e$-transformed urine 23S-pentol. The planned sample size is expected to provide at least 90% power at the final analysis (approximately 12 patients; 2-sided nominal $\alpha = 0.0436$).

For the proportion of patients requiring rescue medication (secondary endpoint), the planned sample size of approximately 12 patients at the final analysis is expected to provide at least 85% power with two sided nominal $\alpha = 0.0436$. This assumes that 85% of patients (approximately 10/12) on placebo and 15% of patients (approximately 2/12) on CDCA will need rescue treatment, with a discordance of 80%, based on McNemar's test.

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Abbreviations used throughout the protocol should not be used by the site when documenting adverse events, medical history, etc. on source documents.

Abbreviation	Definition
AE	Adverse event
AEOI	Adverse event of interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANDA	Abbreviated New Drug Application
API	Active Pharmaceutical Ingredient
AST	Aspartate aminotransferase
AUC_{ss}	Area under the plasma concentration-time curve at steady state
BCVA	Best-corrected visual acuity
BLQ	Below the level of quantification
BM	Bowel movement
BMI	Body Mass Index
BSFS	Bristol Stool Form Scale
$C_{24}H_{40}O_4$	3α, 7α-dihydroxy-5β-cholan-24-oic acid (CDCA chemical formula)
7αC4	7-alpha-hydroxy-4-cholesten-3-one
7α12αC4	7-alpha,12-alpha-dihydroxy-4-cholesten-3-one
CDCA	Chenodeoxycholic acid
CFR	Code of Federal Regulations
CGI-C	Clinician Global Impression of Change
CGI-S	Clinician Global Impression of Severity
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CL/F	Apparent total clearance of the drug from plasma after oral administration
$C_{max,ss}$	Maximum (peak) steady-state plasma drug concentration during a dosage interval
$C_{min,ss}$	Minimum steady-state plasma drug concentration during a dosage interval
COVID-19	Coronavirus Disease 2019
CRO	Clinical Research Organization

Abbreviation	Definition
CTX	Cerebrotendinous xanthomatosis
CV	Coefficient of variation
DB	Double-blind
DB1	Double-blind period 1
DB2	Double-blind period 2
DMC	Data Monitoring Committee
EAS	Enrolled analysis set
ECG	Electrocardiogram
eCRF	Electronic case report form
EEG	Electroencephalogram
EOS	End-of-Study
EOT	End-of-Treatment
ET	Early termination
EU	European Union
FAS	Full Analysis Set
FDA	US Food and Drug Administration
FTT	Failure to thrive
GC-MS	Gas chromatography-mass spectrometry
gCDCA	GlycoChenodeoxycholic acid
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HDL	High-density lipoprotein
HEENT	Head, eyes, ears, nose, and throat
HEP	Hepatitis
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
IRT	Interactive response technology

Abbreviation	Definition
IUD	Intrauterine device
IxRS	Interactive voice/web response system
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDL	Low density lipoprotein
LFT	Liver function test
MAR	Missing at random
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
NA	Not applicable
NIFAS	Newly-initiated Full Analysis Set
NYHA	New York Heart Association
OL	Open-label
OL1	Open-label period 1
OL2	Open-label period 2
PE	Physical Examination
PK	Pharmacokinetic
PK/PD	Pharmacokinetic/Pharmacodynamic
PKAS	Pharmacokinetic Analysis Set
PPAS	Per Protocol Analysis Set
PRO	Patient-reported Outcomes
PSAS	Pediatric Safety Analysis Set
PT	Prothrombin time
RBC	Red blood cell
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SD	Standard deviation
SE	Standard error
SED	Single enzyme defect

Abbreviation	Definition
SOA	Schedule of assessments
SoC	Standard of care
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TBL	Total bilirubin
tCDCA	TauroChenodeoxycholic acid
TEAE	Treatment-emergent adverse event
TEFAS	Treatment-experienced Full Analysis Set
TID	Three times a day
$t_{max,ss}$	Time to reach maximum (peak) plasma concentration following drug administration at steady state
UBA	Urinary bile alcohols
ULN	Upper limit of normal
US	United States
WBC	White blood cell
WHO	World Health Organization
WHO-DD	World Health Organization Drug Dictionary
WOCBP	Women of childbearing potential

4. INTRODUCTION

Cerebrotendinous xanthomatosis (CTX) is an inborn metabolic disorder of bile acid synthesis caused by a single enzyme defect (SED) (sterol 27-hydroxylase). To date, only a few hundred patients with CTX have been identified, but the exact prevalence of CTX is unknown (DeBarber 2014b). Clinical manifestations of CTX occur in multiple organ systems (including enterohepatic, tendon, ocular, and peripheral and central nervous systems) with the onset of neurologic impairment in the second and third decades of life and early death in the fifth or sixth decades of life if the condition goes undiagnosed (Duell 2018; Salen 1975; Salen 1987; Salen 2017; Berginer 1984).

CTX is caused by mutations in the cytochrome *P450 CYP27A1* gene that result in production of a defective sterol 27-hydroxylase enzyme (Salen 2017). This enzyme is critical for the synthesis of bile acids, including chenodeoxycholic acid (CDCA). This deficiency leads to a virtual absence (<5%) of CDCA in bile (Shefer 1975), resulting in an increase in the production of atypical bile acids, bile alcohols (including urine 23S-pentol), and cholestanol.

CTX is associated with elevated levels of cholestanol in the blood and deposition of cholestanol and cholesterol in the brain, tendon xanthomas, and bile (Salen 2017); these abnormalities represent the primary mechanisms for many of the neurological and motor symptoms associated with the disease (Keren 2009).

There is a significant unmet need in CTX in that currently there is no labeled medication that is indicated for treatment of CTX. Over the past 35 years, the understanding of the pathophysiology of CTX has led to the use of CDCA as a direct replacement therapy option. Exogenous administration of CDCA restores the physiologic bile acid pool and provides the necessary feedback to reduce the production of cholestanol and bile alcohols, potentially altering the rate of disease progression (Berginer 2009; Price Evans 2007; Yahalom 2013).

Although Chenodal® (chenodiol) is the standard of care (SoC) in the United States (US) for CTX, it has not received marketing approval for treatment of CTX but is currently supplied under medical necessity (Abbreviated New Drug Application [ANDA] 091019). The chemical name for chenodiol is 3α, 7α-dihydroxy-5β-cholan-24-oic acid ($C_{24}H_{40}O_4$). Additional information on the study medication and nonclinical studies conducted for the approval of Chenix (chenodeoxycholic acid), now marketed under the proprietary name Xenbilox, for the treatment of radiolucent gallstones can be found in the Investigator's Brochure (IB). No prospectively designed clinical study has been conducted for the CTX indication.

4.1. Rationale for Study Design

4.1.1. Adult Cohort

For patients in the adult cohort, this study will be a randomized, double-blind (DB), placebo-controlled, 2-period × 2-treatment crossover trial with rescue medication to assess the efficacy and safety of CDCA in patients with CTX who are ≥16 years of age. The rationale for including adolescents who are at least 16 years of age in the adult cohort is based upon the likelihood that most patients of this age would be prescribed the adult dose of 250 mg 3 times a day (TID). The 50th percentile of weight for males and females at 191.5 months of age as indicated in the Center for Disease Control weight-for-age charts is 60.7 kg for males and 53.8 kg for females

(https://www.cdc.gov/growthcharts/html_charts/wtage.htm). At 15 mg/kg/day, the dose for both males and females in the 50th percentile for weight would be above 750 mg. Additionally, there is past experience with treating pediatric and adolescent patients with the adult dose of 250 mg TID (Berginer 2009; Duell 2018). Most adolescents who are 16 and 17 years old are essentially fully grown, and drug metabolism is more similar to adults than children at this point. Patients in the adult cohort are also followed closely with assessments so any progression of disease or change in biomarkers will be identified quickly during the withdrawal period. Parent or legal guardian consent and patient assent, if in the Investigator's opinion the patient is able to understand and provide assent, will still be required for all patients less than 18 years old regardless of cohort assignment unless otherwise directed by a reviewing Institutional Review Board/Independent Ethics Committee (IRB/IEC).

Approximately 12 male and/or female CDCA-naïve or CDCA-treated patients with CTX meeting all inclusion criteria and none of the exclusion criteria and whose clinical symptoms are stable during the open-label run-in period are planned to be randomized in the study. The randomized withdrawal design is intended to directly assess the impact of withdrawing CDCA treatment on biomarkers and symptoms. Owing to CDCA being the treatment of choice (or SoC) for CTX, treating physicians are extremely hesitant to take patients off CDCA treatment for any period. Extensive consultation with treating physicians and experts also indicates that appropriate measures to protect a patient's well-being and unnecessary progression due to withholding CDCA treatment should be established within the study. A maximal duration of placebo withdrawal of 4 weeks with available CDCA rescue is planned to ensure the safety of participating patients.

The crossover design with 2 double-blind periods allows for patients to be their own controls to account for the heterogeneity of disease manifestations across patients. Multiple measurements are planned to be taken within each period to account for intra-patient variability.

The 8-week open-label periods are intended to establish baseline levels of biomarkers and determine individual patient symptoms and clinical manifestations while on CDCA.

Analyses of bile alcohols reported in the published literature (data on file) showed a mean reduction from baseline to end of treatment (EOT) of 91% with very low variability (standard deviation [SD] = 13.4%). Every individual had a significant reduction in bile alcohol; the smallest reduction was 52.9%. This observation indicates a consistent and dramatic effect of CDCA on reduction of bile alcohols. Reductions in bile alcohols were present after less than 1 month of treatment with CDCA with a mean reduction from baseline to EOT of 94% (SD = 1.042%) observed (n = 4). The effect appeared to be sustained in patients with a treatment duration between >1 month and >48 months.

CDCA reduced cholestanol levels in patients with CTX. Reductions were observed within 1 month of treatment and the reduction was even greater with longer term treatment. Evidence supports the stability of the response with treatment durations of more than 4 years (Koopman 1985).

Patients from the published literature with available baseline cholestanol levels had a mean (SD) baseline cholestanol level of 3.132 (3.4307) mg/dL. At EOT, a mean percent reduction from baseline of 75.3% was observed with low variability (SD = 19.6%). Following treatment initiation, no patients with CTX had an increase in cholestanol levels, while in some patients,

cholestanol levels were undetectable. The only patients whose cholestanol level did not decrease were those with normal cholestanol levels at baseline (Koopman 1985).

Consistent results were observed when data was broken down by treatment duration. After less than a month of treatment, a mean percent reduction from baseline of 64.7% was observed with a low variability (SD = 22.7%). Similar reductions were observed between 1 month and 48 months of treatment and after 48 months or more of treatment with CDCA (approximately 71.7% and 84.5%, respectively). Results suggest that a sustainable change in cholestanol levels could be observed shortly after treatment initiation and occurs independently of age (Koopman 1985).

Serum 7-alpha-hydroxy-4-cholesten-3-one (7αC4) and 7-alpha-12-alpha-dihydroxy-4-cholesten-3-one (7α12αC4) have been proposed as important markers for diagnosis and monitoring of replacement therapy in CTX (DeBarber 2010; DeBarber 2014a; DeBarber 2014b; Bjorkhem 2013). Circulating 7αC4 closely mirrors cholesterol 7α-hydroxylation rate, thus reflecting the activity of the "classic" pathway of bile acid synthesis (Bertolotti 2008). Furthermore, 7αC4 is strictly correlated with the accumulation of cholestanol in the brain in both CTX patients (Panzenboeck 2007) and mice with disruption of sterol 27-hydroxylase (Båvner 2010). In a study of 19 previously untreated CTX patients followed over 4 years, the patients had significantly elevated (from 100-fold to 200-fold increased) plasma concentration of 7αC4 compared to controls ($p<0.01$) (Mignarri 2016). Serum levels of 7αC4 consistently decreased after CDCA treatment; however, 7αC4 never normalized in half of patients, and some degree of accumulation appeared to persist in most patients. A significant positive correlation was found between plasma values of 7αC4 and cholestanol ($\rho=0.78$; $p<0.01$). In addition to 7αC4, 7α12αC4 will also be assessed. 7α12αC4 is an intermediate of bile acid synthesis downstream from 7αC4. Circulating levels of 7αC4 are indicative of regulation of the bile acid synthetic pathway and show a degree of natural diurnal variation with variation observed in individuals without disease. In contrast, 7α12αC4 is almost undetectable in individuals without CTX and marked elevations are seen in patients with CTX. Thus, 7α12αC4 has emerged as a sensitive marker of misregulation of this pathway and is used in the diagnosis of the disease (DeBarber 2014b).

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

- Declaration of Helsinki
- Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

4.1.2. Extension of an Open-Label Period as a Result of COVID-19

In December 2019, a novel coronavirus (SARS-CoV-2) was identified in hospitalized patients in Wuhan, China. Since then, the Coronavirus Disease 2019 (COVID-19) has spread to thousands of international locations, including the United States, and was declared a global pandemic by the World Health Organization in March 2020.

COVID-19 has had a global impact disrupting supply chains, transportation lanes, resource allocation within service providers, and day-to-day operations at health institutions. Successful study operations are dependent on these components – any disruption carries the risk of hindering the ability to properly manage patient safety and negatively impacting the integrity of resulting study data. COVID-19 has forced many health institutions to limit nonessential on-site visitors, consistent with local and national social distancing guidelines. While clinical trial visits are permitted at most institutions, there is risk that visit restrictions may continue, thereby affecting an Investigator's ability to complete study visits and perform necessary safety evaluations.

As the world faces a viral pandemic, it is critical that patient safety is preserved. Since the key risks previously noted may impact the dispensation of blinded and/or open-label rescue medication, an extension to each open-label period will be added to the investigational plan to delay the start of a double-blind withdrawal period until, in the opinion of the Investigator and Medical Monitor, the regional impact of this global health emergency has stabilized and study visits can proceed without interruption. The extension of the open-label periods will also ensure that patients continue to receive open-label study medication while completing appropriate safety monitoring.

The extension visits in an open-label period will occur on a monthly basis, which aligns with the dispensation schedule of open-label study medication. Additionally, the monthly visit schedule reduces the burden placed on patients to travel to a health institution during a viral pandemic. Finally, monthly laboratory assessments are part of the standard of care for patients with CTX who are treated with CDCA.

4.1.3. Pediatric Cohort

For patients in the pediatric cohort, this is a 24-week, open-label study with an 8-week titration period to identify a safe and tolerable dose and a 16-week treatment period at the safe and tolerated dose as maintenance treatment to assess the safety and pharmacokinetics (PK) of CDCA in pediatric cohort patients ≥1 month of age to <16 years of age with CTX. The limit of at least 1 month of age is to allow for molecular genetic sequencing confirmation of the CTX diagnosis.

CDCA-naïve or CDCA-treated pediatric patients with CTX meeting all inclusion criteria and none of the exclusion criteria are planned to be enrolled. The main objective for this part of the study is to explore dosing, exposure, and safety in this important population.

4.2. Rationale for Dose

4.2.1. Adult Cohort

CDCA is currently the SoC in the US for CTX and the proposed dose is 750 mg per day (as 250 mg TID) in adults. These doses were chosen empirically by clinicians based on the need to optimize the absorption of CDCA. Free CDCA is absorbed in the gut, transported to the liver, and then conjugates with glycine and taurine. Glyco-CDCA and Tauro-CDCA conjugates circulate in the enterohepatic circulation. Additionally, dividing the total dose into three portions maximizes the body's ability to absorb CDCA from the gut in adults. Available data indicate that treatment with CDCA 750 mg/day (typically administered as 250 mg TID) or higher is anticipated to result in maximal inhibition of bile acid synthesis. Further information on the rationale for the dose is provided in the IB.

4.2.2. Pediatric Cohort

Doses for pediatric patients are consistent with doses currently prescribed in practice and reported extensively in the literature (Salen 2017). As pediatric patients tend to weigh less than adult patients, their dose is a weight-based dosing regimen at doses up to 15 mg/kg/day TID, as frequently prescribed to pediatric patients (van Heijst 1998).

A weight-based dose titration of 5, 10, and 15 mg/kg based on safety and tolerability followed by treatment at the maximum tolerated dose has been proposed for treatment-naïve pediatric cohort patients who participate in this study. Treatment-experienced pediatric cohort patients who participate in the study will initiate treatment at their current dosing regimen and titrate up to a maximum dose of 15 mg/kg/day TID based on safety and tolerability. Pediatric cohort dosing of CDCA will not exceed an equivalent dose of 750 mg/day.

4.3. Rationale for Comparator Group-Adult Cohort

In patients with CTX, Chenodal is considered a medical necessity and the SoC in the US. There is no other available treatment that is appropriate to use as a comparator in assessing the effects of CDCA treatment in the general CTX population. The use of a placebo over a short duration as a comparator is justified as it presents the most efficient way to evaluate the efficacy and safety of SoC therapy in this withdrawal study, with rescue measures in place to protect patient safety.

5. STUDY OBJECTIVES AND PURPOSE

The purpose of this study is to investigate the efficacy and safety of CDCA in patients with CTX in comparison with placebo.

5.1. Efficacy Objective

- Determine the effects of CDCA, compared with placebo, on biomarkers and clinical symptoms of CTX in adult patients with CTX.

5.2. Safety Objective

- Assess the safety and tolerability of CDCA in adult and pediatric patients.

5.3. Exploratory Objectives

- Characterize the relationship among biomarkers in adult and pediatric patients.
- Characterize steady-state pharmacokinetics (PK) of CDCA and 2 conjugated forms of CDCA, GlycoChenodeoxycholic Acid (gCDCA) and TauroChenodeoxycholic Acid (tCDCA) in adult patients.
- Characterize the relationship between dose and PK concentration among pediatric patients.
- Assess biomarkers and health status among pediatric patients treated with CDCA.

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This study is made up of 2 designs: a randomized, double-blind, crossover study design among patients ≥16 years of age at Screening (adult cohort) and an OL, dose-titration and maintenance study design among pediatric patients ≥1 month and <16 years of age at Screening (pediatric cohort).

6.1.1. Adult Cohort

Patients in the adult cohort will participate in a randomized, double-blind, placebo-controlled, 2-period × 2-treatment crossover study with rescue medication to assess the efficacy and safety of CDCA in the treatment of CTX. Approximately 12 male and/or female CDCA-naïve or CDCA-treated patients ≥16 years of age at Screening with CTX meeting all inclusion criteria and none of the exclusion criteria and whose clinical symptoms are stable during the OL run-in period (open-label period 1 [OL1]) are planned to be randomized in the study. At least 6 of the patients randomized in the study will be treatment-experienced (ie, those who have been on a stable dose of CDCA for at least 2 months prior to the run-in period). Patients who are either CDCA-naïve or those with less than 2 months of treatment prior to the run-in period are considered newly initiated patients.

The study design is summarized in Figure 1.

Patients will be screened at Visit 1 (Screening; Appendix 1) and those who satisfy all the inclusion and none of the exclusion criteria will participate in the 8-week OL1 to establish reliable baseline measurements for biomarkers and other efficacy assessments. Patients who fail screening may be rescreened as needed.

Adult cohort patients (≥16 years of age) will receive 250 mg open-label CDCA TID during the OL periods of the study (ie, OL1 and open-label period 2 [OL2]). During the DB periods (double-blind period 1 [DB1] and double-blind period 2 [DB2]), adult cohort patients will receive either blinded CDCA 250 mg TID or matching placebo TID.

Following the OL1 period, patients will be reassessed for eligibility before randomization (Day 1, Visit 6 [Appendix 1]). To be randomized, patients are required to have tolerated CDCA and not have developed any condition that, in the judgment of the Investigator, warranted discontinuation of CDCA treatment.

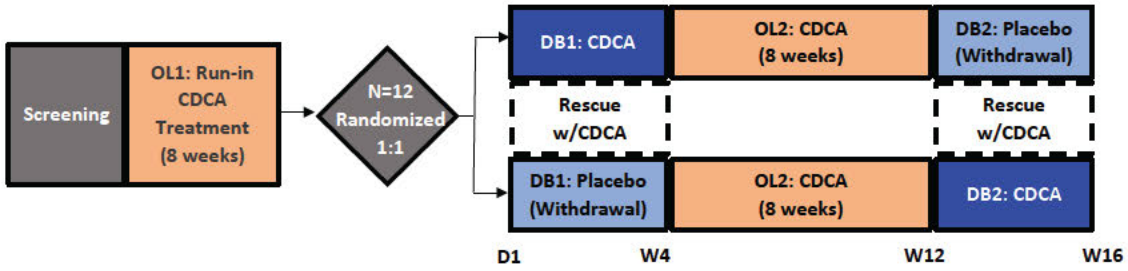
Adult cohort patients will be randomized on Day 1 at a 1:1 ratio to 1 of 2 treatment sequences (AB or BA) to enter a 4-week DB period (DB1), an 8-week OL period (OL2), and then a second 4-week DB period (DB2).

Blinded or open-label 250 mg CDCA TID rescue medication will be provided during the DB periods, if needed, based on blinded biomarkers (blinded rescue) and/or clinical CTX-related symptoms (open-label rescue). Patients who require blinded and/or open-label CDCA rescue medication in a given period will remain on the rescue medication for the remainder of the respective DB period. Patients who require rescue medication during DB1 but otherwise remain eligible should continue to OL2 and then to DB2.

Visits at an alternative site by a trained medical professional (eg, licensed nurse) will be permitted to reduce the travel burden for patients. The visits that qualify for being conducted at an alternative site by a home health vendor are indicated in the Adult Cohort Schedule of Assessments (SOA, Appendix 1).

After the last dose of study medication, patients may transition to treatment for CTX per the guidance of the Investigator or prescribing physician. A safety follow-up phone call will be conducted for all patients 30 (±7) days after last dose of study medication.

Figure 1: Study Design Diagram for Adult Cohort



CDCA = chenodeoxycholic acid; D = day; DB1 = double-blind period 1; DB2 = double-blind period 2; OL1 = open-label period 1; OL2 = open-label period 2; W = Week.

Note 1: Patients will be contacted (via telephone call) 30 (±7) days after the last dose of study medication to ascertain patient safety.

Note 2: Patients randomized to placebo will receive blinded CDCA rescue if biochemical criteria are triggered and open-label CDCA if new or worsening CTX-related symptoms trigger rescue during DB1 or DB2. Patients randomized to CDCA will continue to receive blinded CDCA rescue if triggered by biochemical criteria and will receive open-label CDCA rescue if new or worsening CTX-related symptoms are present.

4-Week DB1: On Day 1, patients will be randomized to treatment sequence AB or BA. Patients randomized to sequence AB will receive blinded 250 mg CDCA TID for 4 weeks or until rescue medication criteria are triggered; patients randomized to sequence BA will receive placebo TID for 4 weeks or until rescue medication criteria are triggered. Should rescue medication criteria be met, patients will receive 250 mg CDCA TID until the end of DB1. Patients will return to the clinic on Day 8, and weekly thereafter for the remaining 3 weeks.

8-Week OL2: Upon completion of DB1, patients will receive open-label 250 mg CDCA TID for 8 weeks and return to the clinic for biweekly scheduled visits.

4-Week DB2: At Week 12, patients who were randomized to sequence AB will crossover into DB2 and receive placebo treatment TID for 4 weeks or until rescue medication criteria are triggered; patients who were randomized to sequence BA will receive blinded 250 mg CDCA TID treatment for 4 weeks or until rescue medication criteria are triggered. Should rescue medication criteria be met, patients will receive 250 mg CDCA TID until the end of DB2. Patients will return to the clinic on Day 92 (7 days after first day of DB2 period), and weekly thereafter for the remaining 3 weeks.

6.1.2. Extension of an Open-Label Period as a Result of COVID-19

An adult cohort patient's participation in an OL period (ie, OL1 or OL2) may be extended to delay the start of a DB withdrawal period (ie, DB1 or DB2) if study and/or site operations are disrupted as a result of the COVID-19 pandemic. The decision to postpone the start of a DB period will be at the discretion of both the Investigator and Medical Monitor. If it is decided to extend an OL period, the patient will return to the study site once a month from the Visit 4 (OL1) or Visit 12 (OL2) date or last dispensation of OL study medication during the respective OL period. During these extension visits, the Investigator will ensure patient safety monitoring and dispensation of additional OL study medication is performed. Efficacy assessments during the extension visits are optional. See Appendix 6 for the Schedule of Assessments.

Patients will continue to receive 250 mg open-label CDCA TID during the extension periods. Details within the clinical study protocol regarding study medication in the OL1 and OL2 periods are applicable for the extension periods. A patient's total duration on treatment may extend beyond 24 weeks if an extension visit is required because of COVID-19.

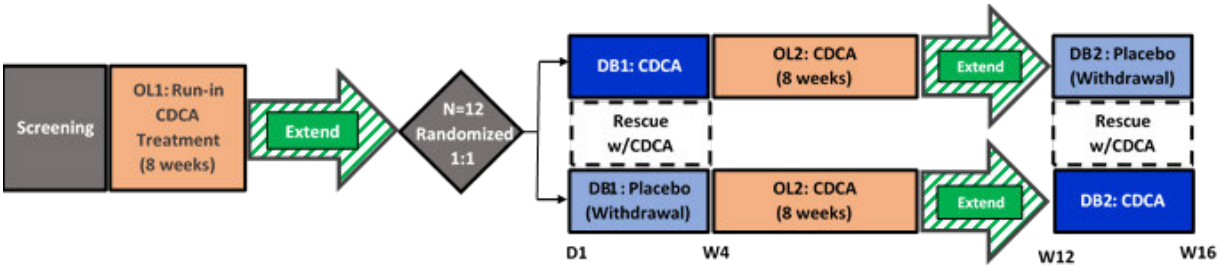
A patient may proceed with the start of a DB withdrawal period when the Investigator and Medical Monitor agree that the impact to site and/or study operations because of COVID-19 has stabilized. The Investigator and Medical Monitor will consider the site's ability to perform study visits without interruption and the timely reporting of necessary study results. A patient will resume the patient schedule as detailed in Appendix 1 when starting a DB period.

If a patient completes 2 or more extension visits in 1 OL period, the patient must complete a study visit 2 weeks prior to the start of a DB period to complete all safety and efficacy assessments that were otherwise required during a visit in the OL period.

Visits at an alternative site by a trained medical professional (eg, licensed nurse) will be permitted to reduce the travel burden for patients. The visits that qualify for being conducted at an alternative site by a home health vendor are indicated in the Schedule of Assessments (Appendix 6).

The modified study design is summarized in Figure 2.

Figure 2: Study Design Diagram for Extension of Open-Label Period in the Adult Cohort



CDCA = chenodeoxycholic acid; D = day; DB1 = double-blind period 1; DB2 = double-blind period 2; OL1 = open-label period 1; OL2 = open-label period 2; W = Week.

6.1.3. Pediatric Cohort

Pediatric cohort patients (≥1 month and <16 years at Screening) will participate in a 24-week, open-label cohort with an 8-week titration period to identify a safe and tolerable dose and a 16-week treatment period at the safe and tolerated dose as maintenance. Patients will be screened at Visit 1 (Appendix 2) and those who satisfy all the inclusion criteria and none of the exclusion criteria will be eligible to participate in this cohort to evaluate safety, PK, and biomarkers in this population. Patients will have visits every week during the titration period and every 4 weeks during the treatment period. A safety follow-up phone call will be conducted for all patients 30 (±7) days after the last dose of study medication.

During the dose-titration period, dose escalation decisions will be based on safety and tolerability (Section 6.5.1).

Dosing will be as follows:

- Treatment-naïve pediatric cohort patients will receive doses of 5 mg/kg/day (TID), 10 mg/kg/day (TID), or 15 mg/kg/day (TID) titrated up every 2 weeks based on safety and tolerability over a period of 8 weeks.
- Treatment-experienced pediatric cohort patients currently taking a dose ≥5 mg/kg/day of CDCA will start at their current dose and titrate up every 2 weeks to the next dose level up to 15 mg/kg/day based on safety and tolerability over a period of 8 weeks. Patients with a history demonstrating intolerance at higher doses will not be required to dose escalate.
- Pediatric cohort dosing of CDCA will not exceed an equivalent dose of 750 mg/day.
- Pediatric cohort patients who are able to take an equivalent weight-based dose of 750 mg/day and are capable of swallowing a tablet will have the option to dose with CDCA 250 mg tablets TID or the liquid suspension form of CDCA.

During the treatment period, dosing will be maintained at the dose tolerated during the titration period. After the last dose of study medication, patients may transition to treatment for CTX per the guidance of the Investigator or prescribing physician.

Figure 3: Study Design Diagram for Pediatric Cohort

D = day; W = Week.

6.2. Number of Patients

Approximately 12 patients ≥16 years of age at Screening are planned to be randomized in the adult cohort.

Pediatric patients ≥1 month and <16 years at Screening will be enrolled separately in the pediatric cohort.

6.3. Study Endpoints

6.3.1. Primary Efficacy Endpoint for the Adult Cohort

- Change from baseline in $\log_e$-transformed urine 23S-pentol at the end of each DB treatment period

6.3.2. Key Secondary Efficacy Endpoints for the Adult Cohort

- Proportion of patients requiring rescue medication during the DB periods
- Percent change from baseline in plasma cholestanol levels at the end of each DB treatment period

- Percent change from baseline in plasma 7αC4 at the end of each DB treatment period

6.3.3. Other Secondary Efficacy Endpoints for the Adult Cohort

- Change from baseline in plasma cholestanol to cholesterol ratio during the DB periods
- Percent change from baseline in plasma 7α12αC4 at the end of each DB treatment period
- Proportion of patients with negative net change in symptoms and manifestations reported in the diary during the DB periods

6.3.4. Safety Endpoints for Both Adult and Pediatric Cohorts

- Change from baseline in body weight, vital signs, physical examinations (PE), electroencephalogram (EEG), 12-lead electrocardiogram (ECG), clinical laboratory parameters (hematology, chemistry, coagulation [adult cohort only], lipid profile [including cholesterol]) and urinalysis laboratory parameters at each visit where parameters are collected
- Incidence of treatment-emergent adverse events (TEAEs), including serious adverse events (SAEs), adverse events (AEs) leading to discontinuation of study medication, and AEs of interest (AEOI)

6.3.5. Exploratory Endpoints for Both Adult and Pediatric Cohorts

6.3.5.1. Adult Cohort Patients

- Percent change from baseline in plasma bile alcohol at the end of each DB treatment period
- Steady-state PK parameters of 3 bile acids that include CDCA, gCDCA, and tCDCA and total CDCA (summed CDCA, gCDCA, and tCDCA). (AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, $t_{max,ss}$, [CL/F])
- Relationships among biomarkers (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4
- Relationships between PK exposure parameters (AUC_{ss}, $C_{max,ss}$, or $C_{min,ss}$) and changes in biomarker levels (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4)
- Relationships between PK exposure parameters (AUC_{ss}, $C_{max,ss}$, or $C_{min,ss}$) and safety endpoints
- Change in disease severity as assessed by the Clinician Global Impression of Change (CGI-C) during each DB treatment period
- Change in health status questionnaire

6.3.5.2. Pediatric Cohort Patients

- Relationships among biomarkers (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4)
- Steady-state PK concentration of CDCA, gCDCA, and tCDCA and total CDCA (summed CDCA, gCDCA, and tCDCA)
- Mean biomarker levels (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4)
- Change in disease severity as assessed by the CGI-C during the treatment period
- Change in health status questionnaire
- Change in developmental milestones

6.4. Treatment Assignment

Adult cohort patients meeting the prerandomization inclusion criteria (Section 7.3) will be randomized on Day 1 in a 1:1 ratio to 1 of 2 treatment sequences (AB or BA) to enter a 4-week DB period (DB1), an 8 week open-label period (OL2), and then a second 4-week DB period (DB2).

Treatment assignment does not apply to the open-label pediatric cohort. All pediatric cohort patients will receive the safe and tolerated dose as determined during the titration period of the study.

6.5. Dose Adjustment Criteria

Dose adjustment for adult cohort patients is not allowed at any point during the study.

6.5.1. Dose Titration in Pediatric Cohort

Dose adjustment during the 8-week dose-titration period in the pediatric cohort will be based on safety and tolerability (Table 1 and Appendix 2).

- Treatment-naïve pediatric cohort patients will receive doses of 5 mg/kg/day (TID), 10 mg/kg/day (TID), or 15 mg/kg/day (TID) titrated up every 2 weeks based on safety and tolerability over a period of 8 weeks.
- Treatment-experienced pediatric cohort patients currently taking a dose ≥5 mg/kg/day of CDCA will start at their current dose and titrate up every 2 weeks to the next dose level up to 15 mg/kg/day based on safety and tolerability over a period of 8 weeks. Patients with a history demonstrating intolerance at higher doses will not be required to dose escalate.
- Pediatric dosing of CDCA will not exceed an equivalent dose of 750 mg/day.

Table 1: Dose Titration Criteria for 8-Week Titration Period in Pediatric Cohort

	Pediatric Cohort Patient	
	Treatment-Naïve	**Treatment-Experienced**
Clinical or Laboratory Observations	Initiate treatment at 5 mg/kg/day	Start at their current dose
• <1.5× ULN of AST, ALT and TBL • and no clinical symptoms	Titrate up to the next dose level every 2 weeks (up to 15 mg/kg/day). Pediatric dosing of CDCA will not exceed an equivalent dose of 750 mg/day.	Titrate up to the next dose level every 2 weeks (up to 15 mg/kg/day) (if already at 15 mg/kg/day, then maintain dose). Pediatric dosing of CDCA will not exceed an equivalent dose of 750 mg/day.
• ≥1.5 to <3× ULN of AST or ALT • or ≥1.5 to <2× ULN TBL	• Maintain at current dose • If asymptomatic, retest one week later • If the elevation(s) returns to baseline, consider dose increase • If the AST and/or ALT elevation(s) remain 1.5 to <3× ULN, and/or the total bilirubin remains ≥1.5 to <2× ULN, then maintain and continue to monitor closely and draw LFTs weekly	• Maintain at current dose • If asymptomatic, retest one week later • If the elevation(s) returns to baseline, consider dose increase • If the AST and/or ALT elevation(s) remain 1.5 to <3× ULN, and/or the total bilirubin remains ≥1.5 to <2× ULN, then maintain and continue to monitor closely for symptoms and draw LFTs weekly
• ≥3× ULN of AST or ALT • or ≥2× ULN TBL • or diarrhea >48 hours	• Temporary Dose interruption • See Section 6.6.1 • See Section 14.2.4	• Temporary Dose interruption • See Section 6.6.1 • See Section 14.2.4

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; CDCA = chenodeoxycholic acid; LFT = liver function tests; TBL = total bilirubin; ULN = upper limit of normal
Note: Patients with a history demonstrating intolerance at higher doses will not be required to dose escalate.

6.6. Interruption or Permanent Discontinuation of Study Medication

6.6.1. Study Medication Interruption

Patients may temporarily interrupt study medication if diarrhea lasts for more than 48 hours or due to elevated liver function tests (LFTs) (Section 14.2.4):

- 3-fold or greater elevations above the upper limit of normal (ULN) of alanine aminotransferase (ALT) or aspartate aminotransferase (AST)
- 2-fold increase in ALT or AST above the baseline value in patients who have elevated values prior to starting study medication
- elevation of total serum bilirubin to ≥2× ULN

If a patient's LFTs are elevated according to any of the above criteria, the patient should be monitored as described in Section 14.2.4. If the diarrhea or LFT elevation(s) resolve(s) as described in Section 14.2.4 and after consultation between Investigator and Medical Monitor, treatment may resume at the same dose for the patients in the adult cohort and at the same dose or an appropriately adjusted dose for the patients in the pediatric cohort. If the LFT elevation(s) or the diarrhea do not resolve, study medication should be permanently discontinued (Section 6.6.2 and Section 14.2.4).

Patients may temporarily interrupt study medication if deemed necessary in the clinical judgement of the Investigator. The patient may restart the study medication at the discretion of the Investigator after consulting with the Sponsor's Medical Monitor.

Patients who temporarily interrupt study medication prior to completion of the study will continue with study visits and assessments according to the schedules of assessments (Appendix 1 and Appendix 2).

6.6.2. Permanent Discontinuation of Study Medication

DB study medication may be discontinued if the adult cohort patient requires rescue with open-label CDCA per Investigator judgment. Patients who discontinue DB study medication prematurely will be asked to remain on the study. In particular, patients who discontinue DB study medication during DB1 should still undergo DB2 unless an eligibility criterion is no longer satisfied.

During the course of this study patients may permanently discontinue study medication for any of the following reasons:

- Any SAE, treatment-emergent AEOI (Section 14.2.4), clinically significant AE, clinically significant laboratory abnormality, intercurrent illness or other medical condition that indicates to the Investigator that continuation on study medication is not in the best interest of the patient
- Investigator discretion
- Patient and/or caregiver decision to discontinue study medication
- Patient pregnancy
- Diagnosis of New York Heart Association (NYHA) Class II-IV Class of Heart Failure
- Other

Patients who permanently discontinue study medication early should be encouraged to continue study visits through Week 16 for the adult and pediatric cohorts for continued collection of safety and efficacy data despite stopping study medication, but may withdraw consent at any time (see Section 6.7).

Patients who agree to continue regularly scheduled study visits will complete the EOT assessments listed in the Schedule of Assessments (Appendix 1 or Appendix 2) as close as possible to the patient's last dose of study medication. The visit data, including the primary reason for discontinuation of study medication, will be recorded on the EOT electronic case report form (eCRF). Subsequent study visit data will be recorded on the visit-specific eCRF.

If the patient completed their last dose of study medication ≥23 days prior to their EOT visit, then patient safety may be ascertained during the EOT visit, and a safety follow-up phone call is not required.

Patients who permanently discontinue study medication should transition to treatment for CTX per the guidance of the Investigator or the prescribing physician, including treatment with appropriate medications, as deemed necessary. Patients permanently discontinuing study medication who are not willing to continue with regular study visits, but who are willing to continue for their information to be used for this study, will be encouraged to remain in the study. The Investigator will contact the Sponsor's designee to determine the best approach based on the patient's situation.

6.7. Discontinuation of the Patient from the Study

Patients are free to withdraw consent and/or discontinue participation in the study at any time without prejudice to subsequent standard of care treatment. A patient's participation in the study may also be discontinued at any time at the discretion of the Investigator or Sponsor. Patients may also be discontinued from the study if the study is terminated (see Section 17.3.5).

Patients may be permanently discontinued from the study for any of the following reasons:

- Death
- Withdrawal of patient consent
- Withdrawal of Parent/Legal Guardian consent
- Adverse events, including:
  - Any SAE, clinically significant AE, severe laboratory abnormality, intercurrent illness, or other medical condition that indicates to the Investigator that continued participation is not in the best interest of the patient
- Investigator decision, including:
  - Investigator determines continued participation is not in the patient's best interests due to occurrence of any medical condition or circumstance that exposes the patient to substantial risk and/or does not allow the patient to adhere to the requirements of the protocol
  - Requirement of prohibited concomitant medication
- Pregnancy
- Protocol deviation, including patient failure to comply with protocol requirements or study-related procedures
- Site terminated by Sponsor
- Study terminated by the Sponsor, US Food and Drug Administration (FDA), or other regulatory authorities (see Section 17.3.5)
- Screen failure (eg, at re-screening prior to starting OL1)
- Lost to follow-up (see Section 6.9)

- Other

In general, patients should be encouraged to remain in the study until they complete the study. A patient who permanently discontinues from the study will complete the ET assessments listed in the SOA (Appendix 1 or Appendix 2) as close as possible to the patient's last dose of study medication or as soon as possible for those patients who had previously completed an EOT visit. The visit data, including the primary reason for premature discontinuation from the study, will be recorded on the early termination (ET) eCRF.

Patients discontinuing from the study will not be replaced.

6.8. Early Termination Visit and Withdrawal Procedures

The end of study for patients completing the study is on Visit 18 both for the adult and pediatric cohorts.

For patients in the adult and pediatric cohorts who are withdrawn from the study prior to completion, all ET procedures will be performed at an ET visit, as indicated in the SOA (Appendix 1 and Appendix 2). In addition to the ET visit, patients who discontinue the study prior to completion will be contacted (via telephone call) 30 (±7) days after the last dose of study medication to ascertain patient safety. If the patient who has discontinued the study prior to completion completed their last dose of study medication ≥23 days prior to their ET visit, then patient safety may be ascertained during the ET visit rather than a safety phone call. The completion of the ET visit will mark the end of study for that patient.

6.9. Lost to Follow-up

The Investigator must make every effort to contact patients who fail to return for scheduled visits so that they will not be declared "lost to follow-up." Patients will be considered "lost to follow-up" only after reasonable, documented attempts to reach the patient prove unsuccessful. These attempts include, but are not limited to, the following:

1. Contact all telephone numbers for the patient and his/her listed contacts (to be collected in the source at the patient's entry into the study), as applicable.
2. Contact the patient's primary care physician, referring specialist, or other healthcare professional, as applicable.
3. Send email, text, and postal mail with certified letters to all the patient's addresses and contacts, as applicable.
4. Review available medical records/notes for details of hospitalizations, clinic visits, or other procedures that may indicate the status of the patient, as applicable.
5. Utilize the internet to search for additional contact information, as applicable.
6. Check local, regional, and national public records to locate the patient or search for mortality status as allowed by law, as applicable.

The information and dates of attempted contact must be recorded in the patient's records and the patient's final status recorded in the appropriate eCRF. Once these actions have been exhausted and documented, the Sponsor or Sponsor's designee should be contacted for additional guidance.

7. SELECTION OF PATIENTS

Eligibility must be confirmed and signed/dated informed consent must be obtained prior to any study-related procedure from the patient or from the parent/legal guardian if the patient is <18 years or mentally incapacitated. Assent must be obtained prior to any study-related procedure from patients who are <18 years old or patients who are mentally incapacitated if, in the Investigator's opinion, the patient is able to understand and provide assent.

7.1. Inclusion Criteria

Male and female patients with a clinical diagnosis of CTX are eligible for this study if they meet all of the following criteria:

1. The patient or parent/legal guardian (as appropriate) is willing and able to provide signed informed consent, and where required, the patient is willing to provide assent, prior to any screening procedures.
2. The patient is ≥1 month of age at the time of signing the informed consent and assent, as applicable.
3. Clinical diagnosis of CTX with biochemical confirmation. For treatment-experienced patients, documented historical confirmation of serum cholestanol and/or bile alcohol is acceptable. For treatment-naïve patients, serum cholestanol and urine 23S-pentol levels will be obtained prior to initiating open-label CDCA.
4. If the patient is currently being treated with CDCA at a dose different than the protocol-specified dose, the patient must be willing to change his or her current dose.
5. With Medical Monitor approval, patients currently taking CDCA and riluzole for the treatment of ataxia are eligible, provided their ataxia is well controlled and they have been on a stable dose of riluzole for a minimum of 6 months.
6. Women of childbearing potential (WOCBP), beginning at menarche, must agree to the use of 1 highly reliable (ie, can achieve a failure rate of <1% per year) method of contraception during the course of the study. Highly reliable contraception methods include stable oral, implanted, transdermal, or injected contraceptive hormones associated with inhibition of ovulation, or an intrauterine device (IUD) in place for at least 3 months. One additional barrier method must also be used during sexual activity, such as a diaphragm with spermicide or male partner's use of male condom with spermicide, during the course of the study. WOCBP are defined as those who are fertile, following menarche and until becoming postmenopausal unless permanently sterile; permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A postmenopausal state is defined as amenorrhea for more than 24 consecutive months without an alternative medical cause; women on hormone replacement therapy must have a documented plasma follicle-stimulating hormone level >40 mIU/mL. All WOCBP must have a negative serum pregnancy test at Screening. NOTE: Prior to menarche, pregnancy testing and contraceptive use is not required. However, the patient and their parent/legal guardian must be advised that, immediately upon menarche, the patient will be required to begin pregnancy testing and initiate contraceptive use. This requirement cannot be avoided.

7. Males must be surgically sterile (more than 3 months post vasectomy) or males and their sexual partners must together agree to the use of medically accepted methods of contraception that are considered highly reliable during the course of the study.
8. The patient and/or his/her parent/legal guardian is willing and able to comply with the protocol.

7.2. Exclusion Criteria

A patient who meets any of the following criteria will be excluded from this study:

1. The patient has a negative genetic sequencing result for CTX.
2. The patient is known to have another malabsorption disorder or confounding inflammatory gastrointestinal condition (eg, irritable bowel syndrome).
3. The patient has been diagnosed with NYHA Class II – IV heart failure.
4. If the patient is receiving anticoagulants and, in the judgment of the Investigator, is not well-controlled.
5. The patient is taking medications that impact bile acid absorption, such as bile acid sequestering agents (eg, cholestyramine, colestipol, aluminum-based antacids).
6. The patient is taking cholic acid medication.
7. The patient is pregnant or lactating or presents with a positive serum pregnancy test at Screening, or a positive urine pregnancy test at the first day of OL1 for adult cohort or Titration Start for pediatric cohort.
8. The patient has been (or is currently) enrolled in a clinical study involving study medication or device within 30 days prior to Screening.
9. The patient has other prior or ongoing medical conditions, physical findings, or laboratory abnormalities that, in the Investigator's opinion, could adversely affect the safety of the patient, make it unlikely that the course of treatment or follow up would be completed, or impair the assessment of study results.
10. The patient has a history of drug or alcohol abuse within the past year that, in the judgment of the Investigator, could affect participation in or adherence to the requirements of the study.
11. The patient has a positive serologic test for hepatitis B virus surface antigen, hepatitis C, or human immunodeficiency virus at Screening.
12. The patient and/or his/her parent/legal guardian, in the opinion of the Investigator, is unable to adhere to the requirements of the study.

7.3. Inclusion Criteria Pre-Randomization for the Adult Cohort

Patients in the adult cohort who meet all inclusion criteria and none of the exclusion criteria for participation into the run-in period **AND**

1. Tolerate CDCA treatment, in the judgment of the Investigator.
2. Have not developed any condition that, in the judgment of the Investigator, warrants discontinuation of CDCA.

Patients who fail screening may be rescreened as needed.

8. TREATMENT OF PATIENTS

8.1. Study Medication

Study medication refers to:

- Open-label CDCA, which is received during the run-in period, open-label period, and for open-label rescue CDCA for the adult cohort and all study medication in the pediatric cohort
- Blinded CDCA, which is received during a double-blind period or as blinded rescue CDCA in the adult cohort
- Blinded placebo in the adult cohort

8.2. Description of Study Medication

Adult cohort patients will receive 250 mg CDCA TID during the open-label periods of the study (ie, OL1 and OL2). During the double-blind periods (DB1 and DB2), adult cohort patients will receive either CDCA 250 mg TID or matching placebo TID based upon their treatment assignment, either AB or BA (Table 2). Those assigned to AB will receive blinded 250 mg CDCA TID during DB1 period and placebo TID during the DB2 period. Those assigned to BA will receive placebo TID during DB1 period and blinded 250 mg CDCA TID during the DB2 period.

Rescue 250 mg CDCA TID (blinded or unblinded) will be administered, if needed, based upon criteria described in Section 8.3.

Pediatric cohort patients will receive open-label CDCA. Pediatric cohort patients who turn 16 years of age while participating in the study will remain in the pediatric cohort and continue dosing with open-label CDCA. Patients who are able to take an equivalent weight-based dose of 750 mg/day and are capable of swallowing a tablet will have the option to take CDCA 250 mg tablets TID or the liquid suspension form of CDCA.

Table 2: Study Medication

	CDCA[a]		Placebo
	Adult Cohort (≥16 years of age)	**Pediatric Cohort (≥1 month and <16 years of age)**	**Adult Cohort (≥16 years of age)**
Product Name	**CDCA**	**CDCA**	**NA**
Dosage Form	Tablet	Liquid suspension or tablet 250 mg TID	Tablet
Dose	250 mg, TID	5 mg/kg/day (TID) 10 mg/kg/day (TID) 15 mg/kg/day (TID) Pediatric dosing of CDCA will not exceed an equivalent dose of 750 mg/day.	TID
Route of Administration	oral	Oral For pediatric cohort patients, the CDCA tablet(s) will be carefully crushed by the pharmacist and added to a sodium bicarbonate solution 8.4% (1 mmol/mL) to create a 10 mg/mL liquid suspension (ie, one 250 mg tablet per 25 mL) and supplied to the patient for oral administration. Patients who are able to take an equivalent weight-based dose of 750 mg/day and are capable of swallowing a tablet will have the option to take CDCA 250 mg tablets TID or the liquid suspension form of CDCA.	Oral
Physical Description	White film-coated 250 mg tablets, imprinted "MP" on one side and "250" on the other		White film-coated tablets, imprinted "MP" on one side and "250" on the other
Manufacturer	LGM Pharma		LGM Pharma

CDCA = chenodeoxycholic acid; NA = not applicable; TID = 3 times daily

[a] Open-label CDCA (pediatric dose titration and treatment periods and open-label periods and open label rescue during the adult cohort) will be distributed in commercial product packaging for Chenodal as bottles of 100 instead of 30 (Section 9.1.1). Chenodal film-coated tablets for oral administration contain 250 mg of chenodiol, which is the non-proprietary name for CDCA

8.3. Rescue Medication Criteria in the Adult Cohort

Blinded or open-label 250 mg CDCA TID rescue medication will be provided during the DB periods, if needed, based on blinded biomarkers (blinded rescue) and/or clinical CTX-related symptoms (open-label rescue). Patients who require blinded and/or open-label CDCA rescue medication in a given period will remain on the rescue medication for the remainder of the respective DB period. Patients who require rescue during DB1 but otherwise remain eligible should continue to OL2 and then to DB2.

Blinded CDCA will be provided to patients who meet the following criterion: increase of 10 times the baseline value for a given period (ie, Visits 6 and 14) in urine 23S-pentol. Blinded CDCA will be assigned through the Interactive Voice/Web Response System (IxRS) at the next

scheduled in-clinic visit. Values entered in the IxRS system will be the geometric mean of the urine 23S-pentol results from each of the first morning void samples provided by the patient per visit timepoint. If the urine 23S-pentol value is below the level of quantification (BLQ), the lowest value in the analytical range will be used either to calculate the geometric mean or entered into the IxRS system. The Investigator and patient will remain blinded to biomarker data, treatment assignment, and if blinded rescue medication criteria are met.

Patients receiving either blinded CDCA or blinded placebo during the DB period will be rescued with open-label CDCA at the discretion of the Investigator if they present with new or worsening CTX-related symptoms relative to their respective pretreatment assessments as defined in the protocol. The Investigator is responsible for evaluating patients to determine if they meet the criteria for rescue medication at each follow-up visit during DB1 and DB2 (Appendix 1).

The specific reasons (see Appendix 4 and Appendix 5) contributing to the Investigator decision and supportive data will be collected in the database. All assessments should continue as indicated in the SOA (Appendix 1).

8.4. Concomitant Medications

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the patient is receiving at the time of enrollment or receives 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

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

If the patient is receiving anticoagulants, the patient should be well-controlled in the judgment of the Investigator to continue receiving study medication.

8.5. Prohibited Medications

Patients currently taking Chenodal® and/or an alternative therapy for CTX are required to stop their medication/therapy prior to the start of study medication in OL1 for the adult cohort or the titration period for the pediatric cohort. Patients may resume or start taking Chenodal® and/or an alternative therapy for CTX after completing the study (ie, EOS visit) or permanently discontinuing study medication.

Patients should not take medications that impact bile acid absorption, such as bile acid sequestering agents (eg, cholestyramine, colestipol, aluminum-based antacids). Patients should not take cholic acid medication.

8.6. Treatment Compliance

The patient must return the container and unused study medication (if any) to the study site for a compliance check at the visits specified in the SOA (Appendix 1 or Appendix 2).

8.7. Randomization and Blinding for the Adult Cohort

Adult cohort patients will be randomized on Day 1 (Visit 6) at a 1:1 ratio to 1 of 2 treatment sequences (AB or BA). Patients randomized to sequence AB will receive blinded CDCA TID for 4 weeks or until open-label rescue medication criteria are triggered; patients randomized to sequence BA will receive placebo TID for 4 weeks or until either the blinded and/or open-label rescue medication criteria are triggered.

The patient's treatment allocation will remain blinded to all parties involved with the study throughout its course, with the exception of the Data Monitoring Committee (DMC) (see Section 17.1), study medication supply, the SAE reporting contact, Unblinded Study Monitor, and the independent statistical team(s) supporting the DMC. The randomization schedule for treatment allocation will be securely maintained and will not be disclosed until after database lock. Doses allowed during the study are shown in Table 2, and dose modifications are not allowed (Section 6.5).

For emergency unblinding only, randomization codes and corresponding treatment assignments will be made available to the Investigator through the interactive response technology (IRT) system. When possible, the Medical Monitor should be consulted if a medical emergency necessitates unblinding (ie, in situations where knowledge of the unblinded treatment is necessary for further medical management of the patient). If it is not reasonable to inform the Medical Monitor in advance of unblinding, the Investigator must promptly document the case in the patient's study record. Subsequently, the Investigator should contact the Medical Monitor to explain any premature unblinding of treatment assignment (eg, accidental unblinding or unblinding due to an SAE). Procedures for unblinding a patient's treatment will be provided separately to the Investigator. Similarly, if the Medical Monitor breaks the blind to evaluate an emergent safety issue or for regulatory reporting purposes, the Medical Monitor will document within study correspondence the rationale, circumstances, and the person(s) being informed about the unblinding.

Access to randomization codes and corresponding treatment assignments will also be made available through the IRT system to the appropriate, named individual(s) responsible for unblinding suspected, unexpected serious adverse reactions (SUSARs) for reporting to regulatory authorities.

9. STUDY MEDICATION MATERIALS AND MANAGEMENT

9.1. Chenodeoxycholic Acid

The chemical name of CDCA (Chenodal, chenodiol, tablets 250 mg) is 3α, 7α dihydroxy-5β cholan-24-oic acid.

9.1.1. CDCA Packaging and Labeling

CDCA is provided as a film-coated tablets for oral administration that contain 250 mg of CDCA.

Inactive ingredients: pregelatinized starch; silicon dioxide; microcrystalline cellulose; sodium starch glycolate; and magnesium stearate; the thin-film coating contains: opadry YS 2 7035 (consisting of methylcellulose and glycerin) and sodium lauryl sulfate.

CDCA drug substance (or Active Pharmaceutical Ingredient [API]) is synthesized under current Good Manufacturing Practices (GMP) by Erregierre (S.P.A.) Via Francesco Baracca, 19, 24060 San Paolo D'argon BG, Italy.

CDCA 250 mg (tablets) is available as white film-coated 250 mg tablets, manufactured by LGM Pharma, 17802 Gillette Ave., Irvine, CA 92614, imprinted "MP" on one side and "250" on the other. Blinded CDCA will be provided in bottles of 30. Open-label CDCA (pediatric dose titration and treatment periods and open-label periods and open-label rescue during the adult cohort) will be distributed in commercial product packaging of Chenodal as bottles of 100 instead of 30, NDC 68974 876-40. Chenodal tablets for oral administration contain 250 mg of chenodiol, which is the non-proprietary name for CDCA.

9.1.2. CDCA Storage

CDCA must be stored at 20°C to 25°C (68°F to 77°F), in a secure location with limited access at all times.

9.1.3. CDCA Preparation

Not applicable for adult cohort. For pediatric cohort, see Section 9.4 and refer to the Pharmacy Manual.

9.1.4. Blinded CDCA and Open-Label CDCA

Open-label and blinded CDCA are the same drug product. Blinded CDCA study medication will be provided in bottles that are the same as placebo study medication. Open-label CDCA study medication will be provided in commercial product packaging for Chenodal, which contains 250 mg of chenodiol (the non-proprietary name for CDCA).

9.2. Placebo in Adult Cohort

Placebo study medication is provided in the same form as blinded CDCA, as white film-coated 250-mg tablets with no CDCA, manufactured by LGM Pharma, 17802 Gillette Ave., Irvine, CA 92614, imprinted "MP" on one side and "250" on the other. Placebo will be provided in bottles of 30 and will be labeled and handled in the same manner.

9.3. Blinded CDCA and Placebo Administration in Adult Cohort

Blinded CDCA or placebo is administered orally at home. Patients ≥16 years of age will receive 250 mg blinded CDCA or placebo TID. On days of study site visits, patients should hold the first administration of study medication until on site.

9.4. Open-Label CDCA Administration in Pediatric Cohort

For pediatric cohort patients (≥1 month and <16 years of age), the CDCA tablet will be carefully crushed by the pharmacist and added to a sodium bicarbonate solution 8.4% (1 mmol/mL) to create a 10 mg/mL liquid suspension (ie, one 250-mg tablet per 25 mL) and supplied to the patient for oral administration (Table 2). Pediatric cohort patients who are able to take an equivalent weight-based dose of 750 mg/day and are capable of swallowing a tablet will have the option to take CDCA 250 mg tablets TID or the liquid suspension form of CDCA.

On days of study visits, patients should hold the first administration of study medication until after completing visit assessments.

Study medication may be transported directly to a patient via a traceable courier and/or shipping provider to ensure uninterrupted treatment, if necessary. Before doing this, sites will be instructed to acquire verbal agreement from the patient or caregiver, verification of correct shipping address, and the patient's or caregiver's availability to receive the shipment. Confirmation of receipt and the shipping process will be documented in the site source documentation. The patient's identity and personal information will continue to be kept confidential and will not be shared with the Sponsor. Refer to the Pharmacy Manual for additional details.

9.5. Study Medication Accountability

Blinded and open-label CDCA and placebo will be stored at each study site pharmacy, which will be locked with restricted access. No additional procedures are required for the safe handling of the tablets.

The storage temperature in the study site pharmacy where CDCA and placebo are stored must be recorded by using a minimum/maximum thermometer or electronically, 24 hours a day, with printouts available. Any deviation from the recommended storage conditions should be immediately reported to the Sponsor or Clinical Research Organization (CRO).

Recommendations for study participants: the tablets should be kept in the original packaging (bottles). The bottles should be stored in the study participant's home at room temperature (20°C to 25°C [68°F to 77°F]) and out of reach of children and away from direct sunlight. No study medication should be given to other persons.

If necessary because an on-site visit by the patient is not feasible, CDCA or placebo bottles may be transported directly to a patient via courier to ensure uninterrupted treatment. It is the responsibility of the Investigator to document this process in the patient's source documents. Refer to the Pharmacy Manual for additional details.

9.6. Blinded CDCA and Placebo Handling

Blinded CDCA and placebo will be dispensed in 30-count HDPE bottles through a validated randomization and study supply management system. Details are provided in the Pharmacy Manual.

9.7. Study Medication During the Adult and Pediatric Open-Label Periods and Adult Open-Label Rescue Period

During the open-label period of the adult cohort and if a patient receives open-label rescue, CDCA will be provided in commercial product packaging for Chenodal. All study medication in the pediatric cohort will be provided in commercial product packaging for Chenodal. Additional details are provided in Section 9.1 and the Pharmacy Manual.

10. STUDY PROCEDURES

- Study procedures and their timing are summarized in the SOA (Appendix 1 or Appendix 2). Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the Investigator, Medical Monitor, and Sponsor immediately upon occurrence or awareness to determine if the patient should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SOA (Appendix 1 or Appendix 2), is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential patients meet all eligibility criteria. The Investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Study procedures related to the extension of the open-label period because of COVID-19 and their timing are summarized in the Schedule of Assessments (Appendix 6).
- If an on-site visit is not feasible, investigators may interface with patients via telephone or video conferencing.

10.1. Study Visits in the Adult Cohort

The adult cohort will be approximately 24 to 28 weeks in duration. The adult cohort includes a screening period, an open-label run-in period (OL1), the first DB period (DB1) followed by an OL period (OL2), and then a second DB period (DB2) (Figure 1). The duration of the adult cohort may extend beyond 28 weeks if the start of a double-blind period is delayed due to COVID-19.

The screening period is up to 28 days (4 weeks) before OL1 begins. The screening period begins when a patient undergoes a screening assessment after signing informed consent. OL1 lasts for 8 weeks with biweekly study visits. Randomization occurs on Day 1, which is the first day of DB1. During DB1, patients will visit the study site on a weekly basis for 4 weeks. OL2 lasts

8 weeks and patients will visit the site biweekly. DB2 lasts 4 weeks and patients will visit the site on a weekly basis during this period. See Appendix 1 for visit details. See Section 11 for screening assessments details.

If necessary, the extension of the open-label periods as a result of COVID-19 will incorporate monthly visits anchored from the last dispensation of open-label study medication during OL1 or OL2. If a patient completes 2 or more extension visits in 1 open-label period, the patient will complete a visit 2 weeks prior to the start of a double-blind period.

A safety follow-up phone call will be conducted for all patients 30 (±7) days after last dose of study medication.

10.2. Study Visits in the Pediatric Cohort

The pediatric cohort will be approximately 24 to 28 weeks in duration. The pediatric cohort includes an up to 28-day (4-week) screening period; an 8-week, open-label, dose-titration period; and a 16-week, open-label treatment period at the identified safe and tolerated dose as maintenance. The screening period begins when a patient undergoes a screening assessment after signing informed consent. During the titration period, pediatric cohort patients will have weekly visits. During the treatment period, the pediatric cohort patients will return to the study site every 4 weeks. See Appendix 2 for visit details. See Section 11 for screening assessments details.

A safety follow-up phone call will be conducted 30 (±7) days after last dose of study medication.

11. SCREENING ASSESSMENTS

Laboratory samples required at Screening may be collected across multiple days to reduce blood volume drawn on a single day. Laboratory samples at Screening do not have to be collected in fasting conditions. Screening laboratory results must be received before OL1 Week 1 in the adult cohort and before Titration Start in the pediatric cohort. If screening results are not available prior to the end of the patient's 28-day screening period (adult and pediatric cohorts), the patient's screening may be extended up to 2 additional weeks with approval by the Medical Monitor. If a patient's screening window is extended, the patient is not required to repeat screening assessments.

Screening assessments for the adult and pediatric cohorts will be collected according to the respective SOA (Appendix 1 or Appendix 2).

Clinical chemistry, hematology, thyroid stimulating hormone, serologic and coagulation (adult cohort only) tests, as well as routine urinalysis will be conducted and are listed in Appendix 3. For WOCBP, a serum pregnancy test will be conducted. In addition to the urinalysis conducted at the study site, first morning void on 3 mornings within 5 days prior to the visit will be collected and brought to the site visit for urine 23S-pentol analysis. Alternatively, the patient may bring these urine samples to the site after the Screening visit and before the first day of the OL1 period (adult cohort) or start of the titration period (pediatric cohort).

Pediatric cohort patients without bladder control will have urine collected via sterile bags.

A 12-lead ECG will be conducted.

Patients will be trained on completing the Patient Reported Outcomes diary (Appendix 4).

All procedures and assessments will be conducted according to the SOA for the respective cohort (Appendix 1 or Appendix 2) and the Study Manual.

12. ASSESSMENT OF EFFICACY OR DISEASE PROGRESSION

12.1. Biomarker Efficacy Assessments

Laboratory assessments at each visit should be collected prior to the first daily dose of CDCA.

12.1.1. Urine 23S-Pentol

Urine 23S-pentol will be determined using first morning void urine samples and will be calculated as the geometric mean of 3 first morning void urine samples collected within 5 days prior to each visit at which urine 23S-pentol is assessed according to the SOA (Appendix 1, Appendix 2, or Appendix 6). Total (free and glucuronidated) 23S-pentol in urine will be monitored using positive mode liquid chromatography-tandem mass spectrometry (LC-MS/MS) and will be conducted at a central laboratory (Van Grouw 2019).

In the adult cohort, the baseline value for the first DB period is the measurement taken for Day 1; the baseline value for the second DB period is the measurement taken at Day 85 at the beginning of the second DB period.

Pediatric cohort patients who do not have bladder control will have urine samples collected via sterile collection bags.

If necessary because of restrictions related to COVID-19, a patient may transport the first morning void urine samples to the site via courier. It is the responsibility of the Investigator to document this process in the patient's source documents.

12.1.2. Plasma Bile Alcohols (plasma tetrol-glucuronide)

Plasma tetrol-glucuronide will be monitored using LC-MS/MS (DeBarber 2018). Plasma samples will be collected at timepoints as indicated by the SOA (Appendix 1, Appendix 2, or Appendix 6). LC-MS/MS will be conducted at a central laboratory. In the adult cohort, the baseline value for the first DB period is the measurement taken for Day 1; the baseline value for the second DB period is the measurement taken at Day 85 at the beginning of the second DB period.

12.1.3. Plasma 7αC4 and 7α12αC4

Plasma 7αC4 and 7α12αC4 will be monitored using LC-MS/MS (Donato 2018; DeBarber 2018). Plasma samples will be collected at timepoints as indicated by the SOA (Appendix 1, Appendix 2, or Appendix 6). LC-MS/MS will be conducted at a central laboratory. In the adult cohort, the baseline value for the first DB period is the measurement taken for Day 1; the baseline value for the second DB period is the measurement taken at Day 85 at the beginning of the second DB period.

12.1.4. Plasma Cholestanol

Total (free and ester) cholestanol in plasma will be monitored using LC-MS/MS (Xu 2013). Samples will be collected at timepoints as indicated by the SOA (Appendix 1, Appendix 2 or Appendix 6). LC-MS/MS will be conducted at a central laboratory. In the adult cohort, the baseline value for the first DB period is the measurement taken for Day 1; the baseline value for the second DB period is the measurement taken at Day 85 at the beginning of the second DB period.

12.2. Assessments in the Patient Diary

All adult cohort patients (regardless of their history of CDCA use) and/or their caregivers will be asked to complete the daily and per-event patient-reported outcome (PRO) diaries for bowel function and seizures during the 7 days prior to Visit 4 (OL1), Visit 6 (DB1), and Visit 14 (DB2) and throughout the duration of each double-blind period. Additionally, adult cohort patients who are treatment-nave or have been treated with CDCA for ≤2 months prior to screening will also complete the daily and per-event PRO diaries for bowel function and seizures during the 7 days prior to Visit 3 and Visit 5 (OL1).

Pediatric cohort patients (regardless of their history of CDCA use) and/or their parent/legal guardian will be asked to complete the daily and per-event PRO diaries for bowel function and seizures during the 7 days prior to Visits 4, 10, 14, and 18.

Patient and/or parent/legal guardian and/or caregivers will receive periodic reminder notifications about completing the diary as described in the Study Manual. Diaries will be reviewed for compliance at study visits as indicated in the SOA (Appendix 1 or Appendix 2); patient and/or parent/legal guardian and/or caregivers will be retrained as needed on the completion of the diary. The assessments are presented in Appendix 4. Additional information on training and completion of the diary is in the Study Manual.

The primary intent of these assessments is to document CTX-related symptoms including gastrointestinal symptoms and stool consistency (Bristol Stool Form Scale [BSFS]) (Lewis 1997; Lane 2011) and seizures, as well as manifestations for each patient to aid the Investigator in evaluating potential disease progression. A composite endpoint will also be derived from these assessments to aid in evaluating treatment effect at the patient level in the adult cohort.

12.3. Assessments Performed at Clinic Visits

The following assessments and instruments will be conducted at the clinic to document CTX-related symptoms and manifestations to aid the Investigator in evaluating potential disease progression in each patient. While results of these assessments are not necessarily considered efficacy endpoints due to the heterogenous nature of clinical manifestations across CTX patients, composite endpoints may be derived from these assessments or instruments in the adult cohort.

12.3.1. Clinician Global Impression Scale

The Clinician Global Impression (CGI) scale is comprised of the CGI-S (severity) and CGI-C (change) scales. The CGI-S measures the severity of the patient's disease using a global, 7-point scale. The CGI-C is a global, 7-point scale used to assess how much the patient's illness has improved or worsened relative to the patient's condition since baseline. The score ranges from

1 (very much improved) to 7 (very much worse). The CGI-S and the CGI-C will be completed by the clinician at the timepoints indicated in the SOA (Appendix 1 or Appendix 2). Procedures for the CGI-S and the CGI-C are described in the Study Manuals.

12.3.2. Health Status Questionnaire

Patients will complete a visual analog scale by rating their overall health status over the past week from 0 to 100. The health status questionnaire will be completed at timepoints indicated in the SOA (Appendix 1 or Appendix 2).

12.3.3. Motor Developmental Milestones (Pediatric Cohort Patients)

Assessment of motor developmental milestones for pediatric cohort patients will be done by the clinician at the timepoints indicated in the SOA for the pediatric cohort (Appendix 2). The 6 developmental milestones are based upon the World Health Organization (WHO) assessment from the Multicentre Growth Reference Study (Wijnhoven 2004). The clinician will assess whether the milestone is met, not met, or not assessed/not age appropriate. After the baseline assessment at Titration start, there will be a trigger question to ask if there have been any changes to previous milestones assessed (either new milestone met or a regression). Procedures for the Motor Developmental Milestones are described in the Study Manuals.

12.4. Documentation of Rationale for Rescue Treatment Based Upon Investigator's Clinical Judgment

At the end of each clinic visit during the double-blind treatment period, the Investigator will assess if the patient needs rescue treatment to prevent potential irreversible disease progression. The Investigator will review relevant information, including data from the patient's diary, CGI-C, and EEG (Section 12.3). If in the Investigator's judgment, the patient needs rescue treatment, the Investigator will complete a questionnaire intended to document the need for rescue with open-label CDCA, including the specific reasons contributing to the decision (Appendix 5).

13. PHARMACOKINETIC ASSESSMENTS

13.1. Adult Cohort

A blood sample for plasma PK will be drawn within 1 hour prior to study medication administration. PK samples will be collected predose, and at 0.5, 1, 2, 3, 6, and 8 hours postdose at the 6-week visit during the OL1 period as indicated in the SOA (Appendix 1). Patients will need to hold the first dose of study medication for on-site administration.

A standardized low-fat meal will be provided 45 to 60 minutes prior to the first dose of study medication at this visit. The timing of PK sample draws and the administration of study medication relative to the completion of this and subsequent meals are described in Table 3.

Table 3: Timing of Standardized Low-fat Meal, Pharmacokinetic Sample Draws, and Study Medication Administration for the Adult and Pediatric Cohorts

Event/Collection	Adult Cohort	Pediatric Cohort	Window
Standardized Low-fat Meal #1	X	X	Administer 45-60 minutes prior to dosing Complete meal within 30 minutes of starting
Predose Collection	X	X	15-30 minutes post completion of meal
Dose #1	X	X	Immediately after predose collection
0.5 hours post dose #1	X		± 5 minutes
1 hour post dose #1	X	X	± 10 minutes
2 hours post dose #1	X		± 10 minutes
3 hours post dose #1	X	X	± 10 minutes
Small Snack (optional)	X		Complete snack within 30 minutes post 3-hour timepoint
6 hours post dose #1	X		± 15 minutes
Standardized Meal #2	X		Administer 45-60 minutes prior to 8-hour timepoint Complete meal within 30 minutes of starting
8 hours post dose #1	X		15-30 minutes post completion of meal
Dose #2	X		Taken after the 8-hour collection

The PK sample will be analyzed for determination of three bile acids that include CDCA and two conjugated forms of CDCA, gCDCA, and tCDCA.

The PK analysis will be the individual analysis for CDCA, gCDCA, tCDCA, and total CDCA (summed of CDCA, gCDCA, and tCDCA). PK will be analyzed unadjusted for baseline endogenous levels.

PK assessments will include:

- Steady-state PK parameters of CDCA, gCDCA, and tCDCA and total CDCA via noncompartmental PK analysis (AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, $t_{max,ss}$, CL/F)
- Relationships between PK exposure parameters (AUC_{ss}, $C_{max,ss}$, or $C_{min,ss}$) and changes in biomarker levels (cholestanol, bile alcohols, 7αC4 and 7α12αC4, cholesterol)
- Relationships between PK exposure parameters (AUC_{ss}, $C_{max,ss}$, or $C_{min,ss}$) and safety endpoints

13.2. Pediatric Cohort

Patients will need to hold first dose of study medication for on-site administration. Steady-state PK concentrations (CDCA, gCDCA, tCDCA, and total CDCA) in pediatric cohort patients will be assessed via samples obtained at predose, 1 hour, and 3 hours postdose at the visits indicated in the SOA (Appendix 2).

A standardized low-fat meal will be provided 45 to 60 minutes prior to the first dose of study medication at visits with PK assessments. The timing of PK sample draws and the administration of study medication relative to the completion of this and the subsequent meal are described in Table 3.

14. ASSESSMENT OF SAFETY

14.1. Safety Parameters

14.1.1. Demographic/Medical History

During the screening period, demographic and medical history data will be collected according to the SOA (Appendix 1 or Appendix 2). Data collected includes age and year of birth, sex, race, and ethnicity; CTX diagnosis history (eg, age of onset, age of first symptoms, age of diagnosis); medical history; medications and therapies within 30 days of signing informed consent.

14.1.2. Vital Signs

Vital signs assessments (to be taken before blood collection for laboratory tests) will be assessed at study visits as indicated in the SOA (Appendix 1, Appendix 2 or Appendix 6) and include blood pressure (systolic and diastolic), heart rate, respiratory rate, and body temperature.

14.1.3. Weight and Height

Body weight and height will be measured and Body Mass Index (BMI) calculated and recorded according to the SOA (Appendix 1, Appendix 2, or Appendix 6) for adult cohort patients.

Body weight, height, and head circumference will be measured at all visits for pediatric cohort patients to be able to determine Failure to Thrive (FTT). Definitions for determining FTT are provided in the Study Manual. BMI will also be calculated.

14.1.4. Physical Examination

A complete PE will include an assessment of the following body systems: abdomen; cardiovascular; ear, nose, and throat; eyes; hair and skin; lymph nodes; mental status; musculoskeletal; neurological; and respiratory.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

Physical examination will be conducted at timepoints indicated in the SOA (Appendix 1, Appendix 2, or Appendix 6).

14.1.5. Electroencephalogram (EEG)

An EEG will be administered at the timepoints indicated in the SOA (Appendix 1 or Appendix 2). Procedures for the EEG are described in the Study Manuals.

14.1.6. Electrocardiogram (ECG)

Triplicate 12-lead ECG will be obtained as indicated in the SOA (Appendix 1 or Appendix 2) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals. ECG to be assessed prior to vital signs and blood sampling for laboratory tests.

After a minimum of 5 minutes of rest in supine position, 3 consecutive recordings should be made in succession, no more than 2 minutes apart. The full set of triplicates should be completed in less than 4 minutes. These ECGs will be read by the Central Reader, the Investigator, or a designee. The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal ECG finding is clinically significant.

14.1.7. Laboratory Assessments

Laboratory assessments at each visit should be collected prior to the first dose of CDCA. Patients will need to hold the first dose of study medication for on-site administration for all visits. Patients will need to arrive to the study site fasted for visits that include blood collections for assessing lipid and PK parameters. The visits where lipid profile (including cholesterol) and PK parameters are assessed are indicated in the SOAs (Appendix 1, Appendix 2, or Appendix 6). A standardized low-fat meal will be provided 45 to 60 minutes prior to the on-site study medication administration at visits with PK assessments.

Laboratory tests will be conducted at a central laboratory. During the titration period of the pediatric cohort, testing at a local laboratory is permitted to facilitate titration review if there are delays to central laboratory reporting (eg, lost or hemolyzed blood sample). In addition to the tests listed in Appendix 3, if the patient is a WOCBP, β-hCG urine and serum tests will be conducted as described in the SOA (Appendix 1, Appendix 2, or Appendix 6). Procedures are described in the Study Manuals. See the SOA (Appendix 1, Appendix 2, or Appendix 6) for the timing and frequency of clinical laboratory tests.

The Investigator must review the laboratory report, document this review, and record any clinically significant 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 the underlying disease, unless judged by the Investigator to be more severe than expected for the patient's condition. Of particular note, LFTs 3 times the ULN are associated with treatment withdrawal (see Section 6.6.1 and Section 14.2.4).

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days after the last dose of study intervention 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 and CRO notified.
- All protocol-required laboratory assessments, as defined in Appendix 3, must be conducted in accordance with the laboratory manual and the SOA (Appendix 1, Appendix 2, or Appendix 6).
- If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory or at the central laboratory require a change in the patient's management or are considered clinically significant by the Investigator (eg, SAE or AE or dose modification), then the results must be recorded as an AE or SAE in the eCRF and if an SAE, an SAE form should be completed as per Section 14.2.

14.2. Adverse Event Reporting

14.2.1. Adverse Event (AE)

An AE is any untoward medical occurrence in a clinical investigation patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (eg, an abnormal laboratory finding), symptom, or disease temporally associated with the use of the medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

All AEs should be recorded and reported.

AEs may include:

- Symptoms described by the patient
- Clinically significant changes in the patient's physical examination or other signs observed by the Investigator or medical staff
- Test abnormalities (laboratory tests, ECG, X-rays, etc.) that reflect a change from baseline and/or that may result in changes in administration of study medication or in an alteration in medical care (diagnostic or therapeutic)
- Conditions present at baseline that have either worsened or recurred following resolution

Note: It is anticipated that some patients will have a worsening of the underlying condition of the disease, such as increasing diarrhea. Any such conditions considered by the Investigator to be worsening of the underlying condition should be reported as AEs. Investigators should also report such AEs as part of the clinical rescue assessment as appropriate (Section 12.4).

The patient will be evaluated for new AEs and the status of existing AEs at each study visit, including screening or washout periods, or at any time contact is made with the patient outside of a scheduled visit. The Investigator may elicit symptoms using an open-ended question, followed by appropriate questions that clarify the patient's verbatim description of AEs or change in concomitant medications.

14.2.2. Serious Adverse Event (SAE)

An SAE is an AE that results in any of the following:

- Death: The patient died as the result of the event.
- Is life-threatening: An AE that places the patient, in the view of the Investigator or the Sponsor, at immediate risk of death from the AE as it occurred, ie, does not include an AE that had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of an existing hospitalization
  Note: Planned or elective hospital admissions for treatment/procedures for a condition/disease that existed prior to signing the informed consent will be recorded in the patient's screening documents and will not be captured as SAEs. If, however, the admission or procedure occurs other than planned due to a worsening of the condition, the event will be recorded as an SAE.
- Persistent or significant disability/incapacity: An AE that results in a substantial disruption of a person's ability to conduct normal life functions.
- Congenital anomaly/birth defect: A congenital anomaly/birth defect that occurs in the offspring of a patient exposed to the study medication.
- Other medically important serious events: An AE that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

14.2.3. Evaluation of Adverse Events/Serious Adverse Events

14.2.3.1. Causality Assessment

Assessment of the relationship between the AE and exposure to the study medication is important for regulatory reporting and assists in the overall analysis of the safety information. For each AE/SAE the Investigator will determine whether, based on available evidence, there is a reasonable possibility that the study medication caused the event. Causal relationship will be classified according to the following criteria:

- Not Related: There is no suspicion of a causal relationship between exposure and the AE.
- Unlikely Related: There is no evidence for a causal relationship between exposure and the AE; however, such a relationship cannot be ruled out.
- Possibly Related: There is some evidence supporting the possibility of a causal relationship between exposure and the AE.
- Related: There is strong evidence that there is a causal relationship between exposure and the AE.

A causality assessment will be provided for each AE/SAE recorded, even if there is only limited information at the time.

Upon receipt of follow-up information, the Investigator may change his/her assessment of causality and amend the AE/SAE report accordingly.

14.2.3.2. Severity

Severity indicates the intensity of the event and should not be confused with seriousness (ie, Section 14.2.2), which is an event outcome applied for the purpose of event classification and regulatory reporting.

The Investigator will assess the severity of all AEs/SAEs as mild, moderate, or severe, based on the following definitions:

- Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate: A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort, but poses no significant or permanent risk of harm to the research participant.
- Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

14.2.3.3. Outcome

Outcome describes the status of the AE. The Investigator will provide information regarding the patient outcome of each AE. Definitions for possible results of an AE outcome include:

- Recovered/Resolved: the event has improved or the patient recuperated
- Recovering/Resolving: the event is improving
- Not Recovered/Not Resolved: the event has not improved or the patient has not recuperated
- Recovered/Resolved with sequelae: the patient recuperated but retained pathological conditions directly resulting from the disease or injury
- Fatal: termination of life as a result of an AE. There should be only one AE marked with this outcome
- Unknown: not known, not observed, not recorded, or refused

14.2.3.4. Action Taken Regarding the Study Medication

The action taken with regard to the study medication in response to the AE will be provided at the time the event is reported. Options for action taken include the following:

- Drug Withdrawn: medication schedule was modified by permanently terminating a prescribed regimen of medication
- Dose Reduced: medication schedule was modified by subtraction, either by changing the frequency, strength, or amount

- Dose Not Changed: medication schedule was maintained
- Drug Interrupted: medication schedule was modified by temporarily terminating a prescribed regimen of medication
- Unknown: not known, not observed, not recorded, or refused
- Not Applicable: determination of a value is not relevant in the current context, for example, if the AE began and ended prior to treatment or after discontinuation of treatment

14.2.3.5. Assessment of Expectedness

The expectedness of an SAE will be determined by the Sponsor in accordance with the Reference Safety Information (which is contained in the IB).

14.2.4. Adverse Events of Interest

An AEOI (serious or non-serious) is one of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor is required.

All AEOIs must be reported to the Sponsor's Medical Monitor within 24 hours of the Investigator's first knowledge of the event, regardless of causal relationship.

Abnormalities in ALT, AST, and total bilirubin are considered AEOIs and must be reported to the Sponsor's Medical Monitor within 24 hours of awareness if one of the following conditions are met:

- The abnormality represents a new elevation in ALT or AST >3 times the ULN, with or without an elevation of total serum bilirubin >2 times ULN; or
- The abnormality represents a 2-fold increase in ALT or AST above the baseline value in patients who had elevated values prior to starting study medication.

In such instances, the following steps should be taken:

- Temporarily discontinue study medication.
- Repeat testing of ALT, AST, liver-specific alkaline phosphatase (ALP), and total bilirubin; to be completed within 48 to 72 hours to confirm the abnormalities.
- If the abnormality is confirmed by repeat results or after consulting with the Sponsor's Medical Monitor:
  - Complete an AEOI Report Form that documents both the liver function test findings and any associated signs or symptoms, and report by email to the SAE contact on the Study Contact Information page of this protocol.
  - Monitor liver enzymes and serum bilirubin 2 or 3 times weekly. The frequency of re-testing can decrease to once weekly or less if the abnormalities stabilize and the patient is asymptomatic.
  - Perform additional testing to evaluate liver function, as appropriate (eg, international normalized ratio [INR] and direct bilirubin).

- Do not resume study medication until monitoring indicates abnormalities have resolved or stabilized.

Patients are not allowed to resume study medication, unless approved by the Medical Monitor, if they have:

- ALT or AST >8 times ULN
- ALT or AST >5 times ULN for more than 2 weeks
- ALT or AST >3 times ULN and total bilirubin >2 times ULN or INR >1.5
- ALT or AST >3 times ULN with symptoms of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5% eosinophils)

Management of such patients should be closely coordinated with the Sponsor's Medical Monitor. In addition to monitoring liver function tests, the Investigator should perform other relevant clinical and laboratory measurements to identify potential causes of the abnormalities (eg, acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; biliary tract disease or exposure to hepatotoxic medications or environmental chemical agents).

Cases of increased liver function tests will always be considered serious (ie, medically important) if they meet both the following criteria:

- Study medication is suspected to have caused hepatocellular injury, generally shown by a confirmed elevation of 3-fold or greater above ULN in ALT or AST; and,
- The ALT or AST elevations are accompanied by a total bilirubin >2 times the ULN or INR >1.5, without initial findings of cholestasis (elevated serum liver-specific ALP)

14.2.5. Reporting Adverse Events and Serious Adverse Events and Adverse Events of Interest

14.2.5.1. Reporting Adverse Events

AEs (including SAEs) will be captured from the time informed consent is signed to the patient's final visit. In addition, AEs (including SAEs) will be collected for all patients via a follow-up phone call 30 (±7) days after the last dose of study medication to assess patient safety.

- AEs will be recorded using appropriate medical terminology. When recording, it is preferable to provide a diagnosis. In the absence of a diagnosis, each sign and symptom will be captured as a unique AE. Sufficient information should be sought to assist the Investigator both in determining the diagnosis and in making a causality assessment.
- Reporting should not be delayed pending receipt of all required information. If information is unavailable at the time of the initial report, the Investigator is expected to follow up until the required information has been obtained.

14.2.5.2. Reporting Serious Adverse Events

The necessity and time requirements for reporting of SAEs to the Sponsor or its designee and/or regulatory agencies are as follows:

- All SAEs will be reported by email to the SAE contact on the Study Contact page of this protocol or by fax to the number in the Investigator Site File within 24 hours of the Investigator's first knowledge of the event, regardless of causal relationship.
- A completed SAE Report Form containing a detailed written description of the event along with available supporting documents (eg, discharge summary, autopsy report, diagnostic test results, etc.) will be provided by email to the SAE contact on the Study Contact page or by fax to the number in the Investigator Site File.
- Additional information that is not available at the time the initial SAE Report Form was completed will be promptly reviewed and provided by email to the SAE contact on the Study Contact page or by fax to the number in the Investigator Site File within 48 hours of receipt. Full supporting documentation should be solicited by the study site even if the SAE occurred at another institution. Such documentation may include copies of relevant patient/hospital records, discharge summaries, laboratory/test results, or autopsy reports.
- If at any time after the patient has completed participation in the study and for 30 days post end of study (EOS), the Investigator or study staff becomes aware of an SAE that they suspect is related to the study medication (see Section 14.2.3.1), the event and any known details will be reported promptly by email to the SAE contact on the Study Contact page or by fax to the number in the Investigator Site File, following the reporting instructions in this section.
- For medical emergencies, the IxRS can be used at any time by the Investigator to notify the Sponsor for unblinding permissions, if deemed necessary to manage the event. For SUSARs, the Sponsor's Pharmacovigilance designee responsible for managing SAEs will access the IxRS to obtain the patient's treatment assignment for the purpose of regulatory reporting. Refer to the study manuals for unblinding procedures.

14.2.5.2.1. Follow-up of Adverse Events and Serious Adverse Events

All AEs will be followed until resolution, until the condition stabilizes, or until completion of the patient's participation or study termination, whichever occurs first.

Serious AEs will be followed until resolution, until the condition stabilizes, or until the Investigator and the Sponsor agree that follow-up is no longer necessary.

All AEs/SAEs documented at a previous visit/contact where event outcome is designated as not recovered/not resolved, recovering/resolving, or unknown will be reviewed by the Investigator at subsequent visits/contacts. SAEs that are ongoing after completion of the last scheduled visit will continue to be followed to determine the final outcome.

Rules for AE/SAE follow-up apply to all patients, to the extent allowed by the patient's consent. The Investigator will ensure that follow up includes further investigations consistent with

appropriate medical management to understand the nature and/or causality of the AE/SAE. The Sponsor, its designee, or regulatory authorities may also request additional information regarding an SAE at any time.

All follow-up information will be promptly reviewed by the Investigator and provided by email to the SAE contact on the Study Contacts page of this protocol or by fax to the number in the Investigator Site File within the specified timelines. Additional AEs/SAEs may be identified during the review of follow-up information and will be processed in accordance with requirements defined throughout Section 14.2.

14.2.5.2.2. Reporting to Regulatory Authorities, Investigators and Institutional Review Boards/Independent Ethics Committees

The Sponsor will ensure that processes are in place for provision of SAEs and Expedited SAE reports (SUSARs) to Regulatory Authorities, Investigators, and IRBs/IECs, as required, within the specified timelines.

The Sponsor will submit SAE and/or SUSAR reports to regulatory authorities and the Investigator as required. In the US, Investigators will report SAEs and SUSARs to their IRB in accordance with applicable Standard Operating Procedures (SOPs) and/or local reporting requirements. In the European Union (EU), the Sponsor or its designee will notify the IEC of any SUSARs.

Investigators will forward copies of the IRB/IEC notification to the Sponsor or its designee, when applicable.

14.2.6. Pregnancy Reporting

Although not an AE in itself, exposure to study medication during pregnancy must be reported; therefore, all pregnancies will be reported via an initial Pregnancy Notification Form. If a patient becomes pregnant during the study, study medication will be immediately discontinued, and pregnancy will be documented as the reason for study medication discontinuation. If a urine pregnancy test is positive, study medication will be immediately discontinued until a serum pregnancy test confirms the result.

If the Investigator suspects that a pregnancy was the result of an interaction between the study medication and the contraceptive method, in addition to the pregnancy, the study medication interaction will also be captured as a separate AE.

The Investigator will report any pregnancy associated with exposure to study medication throughout the study period. When a site becomes aware that a patient is pregnant, they are to contact the Medical Monitor immediately (within 24 hours of the site becoming aware of the event), complete an initial Pregnancy Notification Form, and send the form by email to the SAE contact on the Study Contacts page of this protocol or by fax to the number in the Investigator Site File.

Female patients will be instructed to notify the Investigator immediately if they discover they are pregnant. Male patients will be instructed to notify the Investigator immediately if they discover that their sexual partner is pregnant. In the latter instance, the partner must provide written consent before pregnancy information can be collected.

If the Investigator learns of a report of pregnancy after signing informed consent, the Investigator will complete the Pregnancy forms and submit them to the study contact on the Study Contacts page of this protocol or by fax to the number in the Investigator Site File.

All pregnancies will be followed to outcome (ie, delivery, elective termination, spontaneous abortion). The Investigator will inform the patient that the Sponsor or its designee is required to gather information regarding the course and outcome of the pregnancy after exposure to the study medication. All study-related visits/contacts involving a known pregnancy will include pregnancy status assessment until pregnancy outcome is known. The Investigator should further obtain follow-up information no later than 1 month after the gestational period to obtain maternal/fetal/neonatal outcome and any other relevant information. Upon obtaining pregnancy outcome, the Investigator will complete the Pregnancy Outcome form and submit it to the study contact on the Study Contacts page of this protocol or by fax to the number in the Investigator Site File.

All information related to the pregnancy and its outcome will be assessed for the occurrence of an AE or SAE. Should an AE or SAE occur in the mother or the child, it will be processed per study guidelines. Spontaneous abortions and stillbirths will always be reported as SAEs. If the pregnancy results in the birth of a child, the neonate should be followed for a minimum of 4 weeks postdelivery. In certain cases, it may be necessary to follow up on the long-term outcome of an AE using the Pregnancy Follow-up Form. If needed, an SAE report form will be completed and provided by email to the SAE contact on the Study Contacts page of this protocol or by fax to the number in the Investigator Site File.

15. DATA COLLECTION, QUALITY ASSURANCE, AND MANAGEMENT

15.1. Recording of Data

The study will use eCRFs for data collection wherever possible. Data collection points identified for data entry by the site will be performed by trained site personnel only. The Investigator is responsible for ensuring that all sections of each eCRF are completed promptly and correctly and that the data can be verified against source data. Additional data may be collected directly from contracted organizations as described in the data management plan and/or data transfer plans.

AEs and medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Similarly, prior and concomitant medications and concomitant therapies will be coded using the World Health Organization Drug Dictionary (WHO-DD).

15.2. Data Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, the Sponsor or its designee may conduct a quality assurance audit.

15.3. Data Management

Data management will be performed by a qualified vendor under their SOPs. The Sponsor will provide oversight.

16. STATISTICS

16.1. Sample Size Justification and Power Analysis for Adult Cohort

The hypothesis to be tested is H_0: $\Delta = 0$ against H_a: $\Delta \neq 0$, where Δ is the true paired treatment difference between CDCA and placebo for the change from baseline in $\log_e$-transformed urine 23S-pentol. The planned sample size is expected to provide at least 90% power at the final analysis (approximately 12 patients; two sided nominal $\alpha = 0.0436$). For the sample size calculation, it is assumed that the true average paired treatment difference between CDCA and placebo in change from baseline in the $\log_e$-transformed urine 23S-pentol is -2.4 with a standard deviation no higher than 1.15. In a meta-analysis of the literature data from eleven CTX patients receiving CDCA treatment, the mean reduction from baseline in $\log_e$-transformed urine 23S-pentol was 3.1 with a 1.15 SD. The assumed 2.4 difference between CDCA and placebo is 2 standard errors (SEs) less than the observed 3.1 reduction.

For the proportion of patients requiring rescue treatment (secondary endpoint), the planned sample size of approximately 12 patients at the final analysis is expected to provide at least 85% power with two sided nominal $\alpha = 0.0436$. This assumes that 85% of patients (approximately 10/12) on placebo and 15% of patients (approximately 2/12) on CDCA will need rescue treatment, with a discordance of 80%, based on McNemar's test.

16.2. Analysis Sets

16.2.1. Adult Cohort

Enrolled Analysis Set (EAS): All adult cohort patients who signed informed consent and take at least one dose in the open-label run-in period. This will be used to assess patient characteristics, disposition during the run-in period, and overall safety presentation during the study.

Full Analysis Set (FAS): All adult cohort patients who are randomized and take at least one dose of randomized study medication will be included in the FAS. Patients in the FAS will be analyzed according to randomized treatment assignment. All efficacy analyses will be based on the FAS.

- **Treatment-Experienced Full Analysis Set (TEFAS)**: All adult cohort patients in the FAS who had been on CDCA for at least 2 months prior to the OL run-in period.
- **Newly-Initiated Full Analysis Set (NIFAS)**: All adult cohort patients in the FAS who are either treatment-naïve or had been on CDCA for less than 2 months prior to the OL run-in period.

Per Protocol Analysis Set (PPAS): The PPAS is a subset of the FAS containing adult cohort patients who meet study eligibility requirements, have at least one efficacy measurement in the DB2 treatment period, and have no protocol deviations that might impact the assessment of efficacy measurements. Patients will be analyzed according to randomized treatment sequence assignment. The PPAS will be used for sensitivity analyses relating to efficacy. The type of protocol deviations governing exclusion from the PPAS will be determined prior to breaking the blind and will be detailed in the SAP.

Safety Analysis Set (SAS): All adult cohort patients who are randomized and take at least one dose of randomized therapy will be included in the SAS. The SAS will be used for safety analyses during the double-blind period and will be based upon randomized study medication actually received.

Pharmacokinetic Analysis Set (PKAS): All adult cohort patients in the EAS with evaluable PK samples will be included in the PKAS. The PKAS will be used for summaries of PK concentration and assessment of PK parameters.

16.2.2. Pediatric Cohort

Pediatric Safety Analysis Set (PSAS): All pediatric cohort patients whose parent/legal guardian signed informed consent and received at least one dose of study medication. This will be used to assess patient characteristics, disposition, biomarkers, health status, and overall safety presentation for pediatric cohort patients.

16.3. Demographics and Baseline Characteristics

Demographic and relevant baseline characteristics will be presented and summarized for the EAS, FAS, and PSAS.

16.4. Analysis of Efficacy Endpoints

Efficacy endpoints will first be summarized by descriptive statistics. Continuous variables will be summarized by mean, SD, SE, confidence intervals (CIs), median, quartiles, minimum, and maximum. For parameters analyzed in the $\log_e$-scale such as urine 23S-pentol, geometric mean and % coefficient of variation (CV) will also be presented.

Categorical variables will be summarized by count, percentage, or shift tables (if applicable).

Descriptive statistics will be produced separately for adult cohort and pediatric cohort patients. The following sections describe inferential statistical testing procedures for efficacy endpoints collected during the double-blind period among adult cohort patients.

16.4.1. Analysis of Primary Efficacy Endpoint of Urine 23S-Pentol

The primary analysis of the primary endpoint of change from baseline in $\log_e$-transformed urine 23S-pentol at the end of each DB treatment period will be conducted via a paired t-test comparing CDCA with placebo using the FAS (adult cohort). Log-transformation is performed due to the anticipated wide range of urine 23S-pentol levels among CTX patients. Urine 23S-pentol will be determined using first morning void urine samples and will be calculated as the geometric mean of 3 first morning void urine samples collected within 5 days prior to each visit at which urine 23S-pentol is assessed. The baseline value for the first double-blind period is the measurement taken for Day 1; the baseline value for the second double-blind period is the measurement taken at Day 85 at the beginning of the second DB period. Measurements obtained after initiation of rescue medication will be considered “missing” for purposes of the primary analysis. An appropriate multiple imputation (MI) method will be used to impute missing values and the values after initiating rescue medication, under the assumption of missing at random (MAR). The imputed values, not the actual values obtained after the initiation of the rescue

medication, will be used in the analysis. Further information on imputation of missing data methods and sensitivity analyses will be described in the SAP.

The primary endpoint of change from baseline in $\log_e$-transformed urine 23S-pentol at the end of each DB treatment period will also be analyzed via a mixed effects model. The $\log_e$-transformed urine 23S-pentol measurements during the DB treatment periods prior to initiating rescue medication will serve as the dependent variable. Fixed effects for randomized treatment, sequence, period, baseline value, nominal time (in days within period; categorical), and randomized treatment-by-time interaction will be included. Random effects for patients will be included, for which an unstructured covariance matrix will be assumed. If convergence issues arise, a first order auto-regressive structure will be used. The REPEATED statement will be used to specify the covariance structure associated with the repeated measures. The primary treatment effect estimate will be the contrast between CDCA and placebo randomized treatment-by-time evaluated at 4 weeks.

16.4.2. Analysis of Key Secondary Efficacy Endpoints

The proportion of patients requiring rescue treatment and the corresponding exact 95% CI will be presented for each treatment. Patients who require blinded and/or open-label CDCA during the DB periods will be considered to have met the event (ie, received rescue medication) for the primary analysis. The difference in proportions, the corresponding exact CI, and exact p-values will be calculated and presented using Prescott's method (Prescott 1981). This analysis will be performed on the FAS.

Percent change from baseline in plasma cholestanol levels, and plasma 7αC4 and 7α12αC4 levels at the end of each DB treatment period will be analyzed in a similar way as for the primary endpoint. Since cholestanol is expected to require longer treatment with CDCA to show meaningful changes, the primary analysis for cholestanol will be conducted on the TEFAS to mitigate against the potential impact of the shorter treatment duration prior to the DB period. For plasma 7αC4, the analysis will be based on the FAS.

16.4.3. Analysis of Other Secondary Endpoints

Other continuous efficacy endpoints (such as plasma cholestanol to cholesterol ratio and plasma 7α12αC4) will be analyzed using the same approach described for percent change in urine 23S-pentol. Other binary efficacy endpoints (such as incidence of new or worsening CTX-related clinical manifestations) will be analyzed using the same approach described for the proportion of patients requiring rescue medication.

The proportion of patients with negative net change in symptoms or manifestations (described below) reported in the diary during the double-blind period is a composite endpoint and will be analyzed as a binary efficacy endpoint using the same approach described for the proportion of patients requiring rescue medication. For each symptom or manifestation, the value for each planned visit (ie, Day 1 [baseline], Weeks 1, 2, 3, and 4, for DB1; Day 85 [baseline], Weeks 13, 14, 15, and 16 for DB2) will be based on the results reported in the diary during the 7 days preceding the visit. Data obtained after initiation of rescue treatment will be excluded from the calculations. The following parameters will contribute to the composite endpoint calculated at the planned visits for each patient:

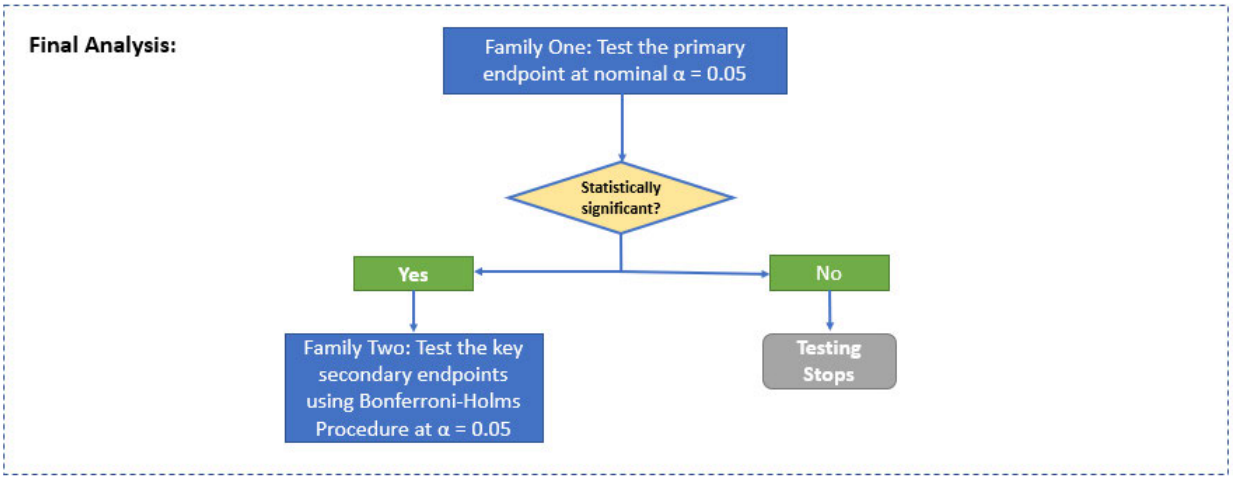
- Bowel function: the proportion of bowel movements with the BSFS scores of 1 (severe constipation), 2 (mild constipation), 6 (mild diarrhea), or 7 (severe diarrhea); the average BSFS score of all reported bowel movements
- Seizures: the rate of seizure per day (ie, total number of seizures/total days with diary data); the average duration of seizures; the average severity of seizures
- Tremors: the proportion of days with tremors (ie, number of days with tremors/total days with diary data); the average number of body parts with tremors; the average severity of all tremors reported
- Individual manifestations: the proportion of days with the specific manifestation (ie, number of days with manifestation/total days with diary data); the weighted (2 for most bothersome manifestations, 1 for other manifestations) average impact or discomfort/distress score (0 to 4, ranging from no impact or discomfort/distress to very severe impact or discomfort/distress)

For each parameter above, the change from baseline score will be calculated as the difference between the value for a specific visit during the double-blind period and the corresponding baseline visit (ie, baseline – postbaseline visit). The sign of the change will be noted as -1, 0, or +1 if it is less than 0, equal to 0, or greater than 0, respectively. Patients are considered to have a negative net change if there are more negative changes than positive changes. Additional details will be described in the SAP.

16.5. Control of Type 1 Error

Multiple comparison procedures will be implemented to strongly control the family-wise type 1 errors across the primary endpoint and the key secondary efficacy endpoints. The first family of hypotheses consists of the analysis of urine 23S-pentol. The second family of hypotheses consist of the key secondary efficacy endpoints of proportion requiring rescue medication, percent change from baseline plasma cholestanol, and percent change from baseline plasma 7αC4 (in no particular order). The schematic of the multiple comparison procedure is presented in Figure 4.

Figure 4: The Multiple Comparison Procedure for Type 1 Error Control



The primary endpoint of urine 23S-pentol will be tested at the final analysis at an $\alpha = 0.05$. If the test is significant, the Family One is rejected and the entire $\alpha = 0.05$ is preserved and then passed onto Family Two of hypotheses (ie, key secondary endpoints) for testing at the final analysis. Otherwise, there is no remaining α to be passed onto Family Two, and therefore, the key secondary endpoints will not be formally tested, and nominal p-values will be reported instead.

The Bonferroni-Holms procedure will be used to control the Type 1 errors for Family Two of hypotheses at an overall $\alpha = 0.05$.

All other secondary efficacy endpoints will be analyzed at the nominal 0.05 significance level.

16.6. Analysis of Safety Endpoints

Descriptive statistics will be used to summarize the safety data by randomized treatment group among adult cohort patients based on the SAS and by administered dose among pediatric cohort patients based on the PSAS.

Clinical laboratory parameters will be measured at baseline and postbaseline visits. Each continuous laboratory variable will be summarized in terms of changes from baseline by treatment group. Laboratory data will also be classified as low, normal, or high relative to the parameter's reference range. Laboratory abnormalities for each treatment will also be summarized using shift tables.

AEs will be coded using the MedDRA by System Organ Class and Preferred Term. AEs that begin after the first administration of study medication, or existing AEs that worsen after the first dose of study medication, are considered TEAEs. The number and percentage of patients reporting TEAEs will be summarized for each treatment group by MedDRA System Organ Class and Preferred Term, by severity, and by relationship to study medication. The number and percentage of patients reporting serious TEAEs, TEAEs leading to treatment discontinuation, and TEAEs of interest (see Section 14.2.4) will also be summarized for each treatment group by MedDRA System Organ Class and Preferred Term.

16.7. Analysis of Pharmacokinetics Endpoints

PK concentration and estimated parameters will be summarized using descriptive statistics.

The PK analysis will be the individual analysis for CDCA, gCDCA, tCDCA, and total CDCA (summed of CDCA, gCDCA, and tCDCA). PK will be analyzed unadjusted for baseline endogenous levels.

16.8. Interim Analysis

No interim analysis will be conducted for this study.

17. SPECIAL REQUIREMENTS AND PROCEDURES

This protocol was designed and will be conducted, recorded, and reported in compliance with the ICH Harmonised Tripartite Guidelines for Good Clinical Practice, 1996; ICH Guidelines for Safety Data Management, 1994; the US Code of Federal Regulations (CFR 21 Parts 50, 56, and 312); and the EU Clinical Trials Directive, 2001/20/EC. The protocol meets legal and regulatory requirements according to the country of conduct.

Institutional and Ethics Review

This protocol and associated Informed Consent Form (ICF), participant information sheet, any information provided to the patient, the Investigator's Brochure, and any proposed advertising material, will be submitted to an appropriate IRB/IEC, applicable regulatory authorities, and host institution(s) for written approval (where applicable). These documents will also be submitted to, and approved by, the above parties for all amendments to the original approved documents (where applicable). Documentation of any applicable approval(s) and the approved ICF will be received by the Sponsor or its designee prior to enrollment of patients and release of study medication.

17.1. Unblinded Data Monitoring Committee

An unblinded DMC, including physicians with experience treating patients with CTX, physicians trained in neurology, internal medicine, and/or pharmacovigilance, as well as statistician(s), will monitor accumulating data, including biomarkers (urine and plasma bile alcohols, plasma cholestanol, plasma 7αC4, and plasma 7α12αC4). The DMC will review efficacy, clinical outcomes, and safety results to potentially stop the study early based on the overall benefit-risk assessment, including the risk of irreversible disease progression among those receiving placebo. The scope of the DMC's review will be further defined within the DMC Charter.

DMC members will not be involved in the study as Investigators or consultants. The DMC will have study conduct oversight as defined by the DMC Charter. All members will have considerable experience with clinical study conduct and DMCs. Any candidate found to have a financial, intellectual, or personal conflict of interest, or whose name is listed on the FDA debarment list, will not be offered membership. Conflicts of interest will be assessed regularly, and if members are found to have a new conflict of interest they will be replaced.

The DMC will meet approximately every 6 months at scheduled meetings but may agree to adjust meeting frequency based upon actual and projected data availability. In addition, ad hoc meetings may be convened, as appropriate, to review safety data.

Based on review of available data, the DMC will advise the Sponsor on the validity and scientific merit of continuing the study.

Written minutes of both open and closed DMC sessions will be prepared. The minutes of closed sessions will be made available to the appointed Sponsor designees only after the database is locked and all data are unblinded.

The DMC may request unblinded individual patient data as appropriate. Data reviewed during the meetings will include, at a minimum, disposition, demographics, exposure, clinical

laboratory results, MedDRA-coded AEs, and AEs leading to early withdrawal of study medication. At each meeting, detailed narratives of interval SAEs (including events resulting in death) are reviewed by the DMC in addition to a cumulative list of all SAEs.

All Investigators and responsible IRBs/IECs will be informed of any decisions made by the Sponsor based on recommendations from the DMC relating to patient safety that alter the conduct of this study. The Investigators will inform patients of such actions, and the protocol and ICF will be revised, as appropriate.

17.2. Changes to the Conduct of the Study or Protocol

Any changes to the study protocol, such as changes in the study design, objectives or endpoints, inclusion and exclusion criteria, and/or procedures (except to eliminate an immediate hazard) will be implemented only after approval by the Sponsor or its designee. All protocol changes will be documented in protocol amendment(s). Protocol amendment(s) (excluding urgent safety amendments) will be signed by the Investigator and approved by the IRB/IEC and regulatory agencies (where required) prior to implementation. Any changes in study conduct that result from a pending amendment will be considered protocol deviations and should be reported to the IRB/IEC. Documentation of IRB/IEC approval will be returned to the Sponsor or its designee.

17.3. Investigator's Responsibilities

The Investigator agrees to:

- Conduct the study in accordance with the protocol and make changes only after receiving written approval from the Sponsor or its designee, except to protect the safety, rights, or welfare of patients.
- Personally conduct or supervise the study.
- Ensure that requirements related to obtaining informed consent and IRB/IEC review and approval comply with ICH E6, CFR 21 Parts 50 and 56, and local laws.
- Report to the Sponsor or its designee any AEs that occur during the study in accordance with ICH E6, CFR 21 Part 312.64, and local laws.
- Read and understand the Investigator's Brochure, including potential risks and side effects of the study medication.
- Ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed of their obligations in meeting the above commitments.
- Maintain adequate records in accordance with ICH E6, 21 CFR Part 312.62, and local laws, and have records available for inspection by the Sponsor, FDA, or other authorized agency.
- Ensure that the IRB/IEC complies with requirements of ICH E6, 21 CFR Part 56, and local laws and will be responsible for initial and continuing review and approval of the clinical study.

- Promptly report to the IRB/IEC and the Sponsor or its designee all changes in research activity and unanticipated problems involving risks to patients or others (including amendments and expedited safety reports).
- Comply with all other requirements regarding obligations of Investigators and all other pertinent requirements listed in ICH E6, 21 CFR Part 312, and local laws.
- Provide progress reports and notifications of SAEs to the IRB/IEC according to local regulations and guidelines.

17.3.1. Patient Informed Consent

Investigators agree to adhere to GCP, which includes ethical principles that have their origin in the Declaration of Helsinki, when developing the patient informed consent and assent (where applicable) forms and when obtaining consent and assent from the patient, parent, or legal guardian. Written informed consent is required prior to enrollment in the study. It is the responsibility of the Investigator to document the consent process within the source documents and obtain consent using an IRB/IEC-approved consent form.

If necessary because of restrictions related to COVID-19, a patient may provide informed consent remotely (ie, via telephone, email, etc.). It is the responsibility of the Investigator to document this consent process within the source documents.

The Investigator will ensure that each patient or parent or legal guardians (as appropriate) is given full and adequate oral and written information about the nature, purpose, possible risks, and benefits of the study as well as potential treatment alternatives. Investigators will ensure that consent/assent is obtained with the appropriate age range form. For developmentally delayed or cognitively impaired patients, Investigators will determine which age range assent will be appropriate based on the patient's physical and cognitive ability and will document the process of determination. Patients or parents or legal guardians (as appropriate) will be notified that they are free to discontinue participation in the study at any time and will be given the opportunity to ask questions and allowed time to consider the information provided.

17.3.2. Case Report Forms

Copies of pertinent records in connection with the study, including all source documents, will be made available to the Sponsor or its designee upon request with due precaution toward protecting the privacy of the patient.

Data will be entered by the site onto the eCRFs in the Electronic Data Capture system or downloaded from a device, as in the case of patient-reported outcomes, or imported into the electronic database as described in the data management plan and/or data transfer plans. Unless explicitly directed, blank data fields are not acceptable. Any erroneous entries made on the eCRFs will be corrected by the site. Changes made to the data after initial entry into the eCRF will be captured via an electronic audit trail and include the reason for change. Incomplete entries or entries needing additional explanation will be queried to the site for clarification.

17.3.3. Record Retention

The Investigator is responsible for oversight and maintenance of the study records and patient source documents. These records will be readily available for audit or inspection.

The Investigator will retain study records for at least 2 years after the last marketing approval has been granted and there are no pending marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of the clinical program. However, these documents should be retained for a longer period, if required by other applicable requirements (eg, applicable local regulatory requirement) or by an agreement with the Sponsor or its designee. The Investigator will contact the Sponsor or its designee prior to any record destruction.

Patient records or other source data will be kept for the maximum period of time mandated by the hospital, institution, or private practice, but not less than 15 years.

If off-site archiving is used, all records will be retrieved and made available for review at the time of an audit or regulatory authority inspection.

17.3.4. Monitoring

A representative of the Sponsor or its designee will visit the Investigator periodically for the purpose of monitoring the progress of this study in accordance with the protocol, GCP, and local regulations. If necessary, because of restrictions related to COVID-19, monitoring activities may be performed remotely. Further details regarding the scope and timing of these remote and on-site monitoring activities are included in the Clinical Monitoring Plan. Non-compliance with the protocol, GCP, and local regulations will be documented and corrective actions implemented, if necessary. It is the responsibility of the Investigator to be present or available for consultation during monitoring visits. During these routine visits, all data pertaining to a patient's participation in this clinical investigation will be made available to the Study Monitor. The Investigator will comply with applicable privacy and security laws for use and disclosure of information. Study Monitors will perform source document verification according to the Clinical Monitoring Plan to ensure consistency between the eCRFs and source documents. Discrepancies will be resolved in accordance with the principles of GCP.

At any time prior to, during, or after completion of the clinical study, an audit may be performed on a study site by the Sponsor or its designee, an IRB/IEC, or a representative of a national regulatory agency. Investigators will notify the Sponsor or its designee upon notification of inspection by a representative of a national regulatory agency. A Sponsor or designee representative will be available to assist in the preparation for study site inspections. All pertinent study data will be made available for verification, audit, or inspection purposes.

An Unblinded Study Monitor will be assigned to the study to perform source data verification of biomarker data entered by central laboratory personnel into the IxRS system (ie, blinded rescue medication calculation) and a standalone biomarker database. Non-compliance with the testing protocols, GCP, and local regulations will be documented and corrective actions implemented, if necessary. The Unblinded Study Monitor will have no other role on the study. Further details regarding the Unblinded Study Monitor are included in the Unblinded Monitoring Plan.

17.3.5. Study or Site Termination

If the Sponsor or its designee, the Investigator, or regulatory authorities discover any conditions during the study that indicate that the study or study site should be terminated, this action may be taken after appropriate consultation between the Sponsor, its designee, and the Investigator. The Sponsor has the right to terminate the participation of either an individual site or the study at any time, for any reason which may include the following:

- The incidence and severity of AEs in this or other studies indicates a potential health hazard to patients.
- Patient enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.
- Investigator(s) do(es) not adhere to the protocol or applicable regulatory guidelines in conducting this study.
- Knowingly false information from the study site is submitted to the Sponsor, its designee, or regulatory authorities.

In the event that the study is terminated early, the Sponsor or its designee will provide specific guidance to study sites regarding the end-of-study procedures.

17.3.6. Study Medication Control

The Investigator will acknowledge that study medication supplies are investigational, and as such must be used strictly in accordance with the protocol and only under the supervision of the Investigator or Sub-Investigator(s) listed on Form FDA 1572 (or regional equivalent). Study medication must be stored in a safe and secure place with limited access and according to Sponsor instructions.

The Investigator must maintain adequate records documenting the receipt and disposition of all study medication supplies. The Sponsor or its designee will supply forms on which to record the date study medication was received and a dispensing record in which to record each patient’s use. It is the Investigator's responsibility to ensure that patients return their unused study medication.

17.3.6.1. Receipt of Study Medication

A proof of receipt, which details the quantity and description of the study medication, will accompany the shipment from the Sponsor or its designee to the Investigator. The Investigator will provide the Sponsor or its designee with a signed and dated copy of this receipt (or an electronic equivalent) within 48 hours after receipt of study medication, while retaining the original within the site pharmacy files. The Investigator is responsible for ensuring that the study medication is maintained in a controlled location, with limited access, and under adequate storage conditions.

17.3.6.2. Disposition of Unused Study Medication

All unused study medication will be maintained under adequate storage conditions in a limited access area. If any unused material is remaining upon completion of the study, the material will be returned to the Sponsor or its designee or destroyed only after the following has been completed:

- Accountability has been performed by a representative of the Sponsor or its designee.
- Appropriate study medication return/destruction documentation has been completed by the study site pharmacist or his/her designee.

17.3.7. Product Handling and Complaints Reporting

If any issues arise during the course of the study related to the quality of the study medication, the study site pharmacist or pharmacy designee will contact the product handling/complaints group listed on the Study Contact page of this protocol.

17.3.8. Insurance

The Sponsor will maintain a liability insurance policy covering all clinical studies under its sponsorship, and that policy will comply with local laws and requirements. The Sponsor or its designee will provide a certificate of insurance to any IRB/IEC, National Competent Authority, or regional Health Authority that may require such a document. Note that this Sponsor insurance coverage does not relieve the Investigator, the Institution, and their collaborators from each maintaining their own liability insurance policy for their clinical research activity.

17.3.9. Data Confidentiality

All patient information obtained during the conduct of the study will be regarded as confidential. Study Monitors, auditors, and inspectors who require access to a patient's medical notes for source document verification will maintain patient confidentiality at all times. An agreement for disclosure of any such information will be obtained in writing and is included in the ICF. No study data will be disclosed to a third party (with the exception of auditors and/or regulatory authorities) without the written consent of the Sponsor. All data shall be secured against unauthorized access.

The Investigator will maintain a list of patient names and identifying information (eg, patient hospital number, unique patient number). This list will not be collected by the Sponsor.

The written participant information sheet will explain that study data will be stored in a computer database, maintaining confidentiality in accordance with national data protection laws. All data that is computer processed by the Sponsor or designee will be identified only by unique patient number/randomization code/patient initials/site number.

When personal patient data are stored in or processed by computer, the data must be protected to prevent their disclosure to unauthorized third parties. Pertinent sections of national data protection laws will be complied with in full, according to the country of conduct.

The ICF will explain that, for data verification purposes, authorized representatives of the Sponsor, a regulatory authority, or an IEC/IRB may require direct access to parts of the hospital or study site records relevant to the study, including patient medical history.

A description of this study will be available on http://www.clinicaltrials.gov, as required by US law. However, no personal patient information will be included on this website.

17.3.10. Clinical Study Report

The Sponsor or its designee is responsible for preparing a clinical study report. If requested, a study summary may be provided to the Investigator.

18. PUBLICATION POLICY

Publication policy is addressed separately in the Clinical Study Agreement.

19. REFERENCES

Båvner A, Shafaati M, Hansson M, Olin M, Shpitzen S, Meiner V, et al. On the mechanism of accumulation of cholestanol in the brain of mice with a disruption of sterol 27-hydroxylase. J Lipid Res. 2010 Sep;51(9):2722-30.

Berginer VM, Gross B, Morad K, et al. Chronic diarrhea and juvenile cataracts: think cerebrotendinous xanthomatosis and treat. Pediatrics. 2009;123(1):143–47.

Berginer VM, Salen G, Shefer S. Long-term treatment of cerebrotendinous xanthomatosis with chenodeoxycholic acid. N Engl J Med. 1984 Dec;311(26):1649–52.

Bertolotti M, Del Puppo M, Gabbi C, Corna F, Carulli L, Pellegrini E, et al. Correlation between plasma levels of 7alpha-hydroxy-4-cholesten-3-one and cholesterol 7alpha-hydroxylation rates in vivo in hyperlipidemic patients. Steroids. 2008 Oct;73(11):1197-202. doi: 10.1016/j.steroids.2008.05.011

Björkhem I. Cerebrotendinous xanthomatosis. Curr Opin Lipidol. 2013;24(4):283-7.

DeBarber AE, Connor WE, Pappu AS, Merkens LS, Steiner RD. ESI-MS/MS quantification of 7alpha-hydroxy-4-cholesten-3-one facilitates rapid, convenient diagnostic testing for cerebrotendinous xanthomatosis. Clin Chim Acta. 2010;411(1-2):43–8.

DeBarber AE, Luo J, Giugliani R, Souza CF, Chiang J (PW), Merkens LS, et al. A useful multi-analyte blood test for cerebrotendinous xanthomatosis. Clin Biochem. 2014a;47(9);860-3.

DeBarber AE, Luo J, Star-Weinstock M, et al. A blood test for cerebrotendinous xanthomatosis with potential for disease detection in newborns. J Lipid Res. 2014b;55(1):146-154.

DeBarber AE, Kalfon L, Fedida A, et al. Newborn screening for cerebrotendinous xanthomatosis is the solution for early identification and treatment. J Lipid Res. 2018;59(11):2214-2222.

Donato LJ, Lueke A, Kenyon SM, Meeusen JW, Camilleri M. Description of analytical method and clinical utility of measuring serum 7-alpha-hydroxy-4-cholesten-3-one (7aC4) by mass spectrometry. Clin Biochem. 2018;52:106-111.

Duell PB, Salen G, Eichler FS, et al. Diagnosis, treatment, and clinical outcomes in 43 cases with cerebrotendinous xanthomatosis. J Clin Lipidol. 2018;12(5):1169-1178.

Keren Z, Falik-Zaccai TC. Cerebrotendinous xanthomatosis (CTX): a treatable lipid storage disease. Pediatr Endocrinol Rev. 2009;7(1):6-11.

Koopman BJ, Wolthers BG, van der Molen JC, Waterreus RJ. Bile acid therapies applied to patients suffering from cerebrotendinous xantomatosis. Clin Chim Acta. 1985;152(1-2):115-122.

Lane MM, Czyzewski DI, Chumpitazi BP, Shulman RJ. Reliability and validity of a modified Bristol Stool Form Scale for children. J Pediatr. 2011;159(3):437–441.

Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997;32(9):920–924.

Mignarri A, Magni A, Del Puppo M, et al. Evaluation of cholesterol metabolism in cerebrotendinous xanthomatosis. J Inherit Metab Dis. 2016;39(1):75-83.

Panzenboeck U, Andersson U, Hansson M, Sattler W, Meaney S, Björkhem I. On the mechanism of cerebral accumulation of cholestanol in patients with cerebrotendinous xanthomatosis. J Lipid Res. 2007;48(5):1167-1174.

Prescott, R.J. The comparison of success rates in cross-over trials in the presence of an order effect. *J R Stat Soc C-Appl*. 1981;30(1):9-15.

Price Evans DA, Salah KA, Mobrad MA, Mitchell WD, Olin M, Eggertsen G. Cerebrotendinous xanthomatosis in a Saudi Arabian family-genotyping and long-term follow-up. Saudi Med J. 2007;28(7):1113-8.

Salen G, Meriwether TW, Nicolau G. Chenodeoxycholic acid inhibits increased cholesterol and cholestanol synthesis in patients with cerebrotendinous xanthomatosis. Biochem Med. 1975;14(1):57-74.

Salen G, Berginer V, Shore V, et al. Increased concentrations of cholestanol and apolipoprotein B in the cerebrospinal fluid of patients with cerebrotendinous xanthomatosis. N Engl J Med. 1987;316(20):1233–1238.

Salen G, Steiner RD. Epidemiology, diagnosis, and treatment of cerebrotendinous xanthomatosis (CTX). J Inherit Metab Dis. 2017;40(6):771-781.

Shefer S, Dayal B, Tint GS, Salen G, Mosbach EH. Identification of pentahydroxy bile alcohols in cerebrotendinous xanthomatosis: characterization of 5beta-cholestane-3alpha, 7alpha, 12alpha, 24xi, 25-pentol and 5beta-cholestane-3alpha, 7alpha, 12alpha, 23xi, 25-pentol. J Lipid Res. 1975 Jul;16(4):280–6.

Van Grouw A, Ganchuluun S, Jeffries KM, Duell PB, DeBarber AE. Quantification of urinary 5β-cholestane-3α,7α,12α,23S,25-pentol as a diagnostic test for cerebrotendinous xanthomatosis (CTX). Poster session presented at: 41st Annual Meeting of the Society for Inherited Metabolic Disorders; 2019 Apr 6-9; Seattle, WA.

van Heijst AFJ, Verrips A, Wevers RA, Cruysberg JRM, Renier WO, Tolboom JJM. Treatment and follow-up of children with cerebrotendinous xanthomatosis. Eur J Pediatr. 1998;157(4):313-316.

Wijnhoven TMA, de Onis M, Onyango AW, Wang T, Bjoerneboe GEA, Bhandari N, et al. Assessment of gross motor development in the Multicentre Growth Reference Study. Food Nutr Bull. 2004;25(1) (suppl 1):S37-45.

Xu Y, Yuan Y, Smith L, Edom R, Weng N, Mamidi R, Silva J, Evans DC, Lim HK. LC-ESI-MS/MS quantification of 4β-hydroxycholesterol and cholesterol in plasma samples of limited volume. J Pharm Biomed Anal. 2013 Nov;85:145-54.

Yahalom G, Tsabari R, Molshatzki N, Ephraty L, Cohen H, Hassin-Baer S. Neurological outcome in cerebrotendinous xanthomatosis treated with chenodeoxycholic acid: early versus late diagnosis. Clin Neuropharmacol. 2013;36(3):78–83.

20. APPENDICES

APPENDIX 1. SCHEDULE OF ASSESSMENTS FOR ADULT COHORT

	V1	V2	V3-5	V6	V7	V8	V9	V10	V11-13	V14	V15	V16	V17	V18
		OL1		DB1					OL2	DB2				
		RIW1, First day	RIW2[a] RIW4[a] RIW6[b]		W1	W2	W3	W4/EOT1[c]	W6[a], 8[a], 10[a]	W12	W13	W14	W15	W16/ EOT2[c] EOS ET
Week/Day	**S**	**D -56**	**D -42 D -28 D -14**	**D1**	**D8**	**D15**	**D22**	**D29**	**D43, D57, D71**	**D85**	**D92**	**D99**	**D106**	**D113**
Visit Window	≤28 days prior to start of OL1	±1 day	±1 day		±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day
Informed Consent/Assent	X													
Inclusion/Exclusion	X			X										
Genetic Testing for CTX[d]	X													
Serologic test for Hep B, Hep C, HIV	X													
Medical History	X													
CTX Medical History[e]	X													
Prior Medication	X													
Demographics	X													
Pregnancy Test[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication		X	X	X	X	X	X	X	X	X	X	X	X	X
Health Status During Prior Week		X		X	X	X	X	X		X	X	X	X	X
CGI-S				X						X				
CGI-C					X	X	X	X			X	X	X	X[g]

	V1	V2	V3-5	V6	V7	V8	V9	V10	V11-13	V14	V15	V16	V17	V18
		OL1		DB1					OL2	DB2				
		RIW1, First day	RIW2[a] RIW4[a] RIW6[b]		W1	W2	W3	W4/EOT1[c]	W6[a], 8[a], 10[a]	W12	W13	W14	W15	W16/ EOT2[c] EOS ET
Week/Day	**S**	**D -56**	**D -42 D -28 D -14**	**D1**	**D8**	**D15**	**D22**	**D29**	**D43, D57, D71**	**D85**	**D92**	**D99**	**D106**	**D113**
Visit Window	≤28 days prior to start of OL1	±1 day	±1 day		±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day
Ophthalmology Exam[h]		X												X
Physical Examination (including body weight)[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Evaluate OL Rescue Medication Criteria[j]					X	X	X	X		X	X	X	X	X
Vital Signs[k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Diary[l]	X[m]		X[n]	X	X	X	X	X		X	X	X	X	X
Urine 23S-pentol (3 first morning voids)[o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis Assessments	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood draw for Cholestanol, 7αC4, 7α12αC4, and Plasma Bile Alcohol	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory Assessments (including coagulation [INR and PT[p]] and LFTs)[q]	X[r]	X[s]	X	X[s]	X	X	X	X[s]	X	X[s]	X	X	X	X[s]
Pharmacokinetic Blood Sample Collection[b]			X[b]											
Adverse Events[t]		Collected continuously												

	V1	V2	V3-5	V6	V7	V8	V9	V10	V11-13	V14	V15	V16	V17	V18
		OL1		DB1					OL2	DB2				
		RIW1, First day	RIW2[a] RIW4[a] RIW6[b]		W1	W2	W3	W4/EOT1[c]	W6[a], 8[a], 10[a]	W12	W13	W14	W15	W16/ EOT2[c] EOS ET
Week/Day	**S**	**D -56**	**D -42 D -28 D -14**	**D1**	**D8**	**D15**	**D22**	**D29**	**D43, D57, D71**	**D85**	**D92**	**D99**	**D106**	**D113**
Visit Window	≤28 days prior to start of OL1	±1 day	±1 day		±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day
EEG[u]		X		X		X		X		X		X		X
12-Lead ECG	X			X		X		X				X		X
Randomization				X										
Study Medication Administration[v]		X	X	X	X	X	X	X	X	X	X	X	X	
Dispense DB Study Medication				X	X	X	X			X	X	X	X	
Dispense OL Study Medication		X	X[w]					X	X[w]					
Medication Accountability			X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: AE = adverse event; BCVA = best-corrected visual acuity; CDCA = Chenodeoxycholic acid; CGI-C = Clinician Global Impression of Change; CGI-S = Clinician Global Impression of Severity; CTX = cerebrotendinous xanthomatosis; D = day; DB = double-blind; DB1 = double-blind period 1; DB2 = double-blind period 2; ECG = electrocardiogram; EEG = electroencephalogram; EOS = end of study; EOT = end of treatment; ET = Early Termination; Hep B = hepatitis B virus surface antigen; Hep C = hepatitis C; HIV = human immunodeficiency virus; INR = international normalized ratio; LFT = liver function test; OL = open label; OL1 =open-label period 1; OL2 = open-label period 2; PK = pharmacokinetics; PT = prothrombin time; PRO = Patient-reported outcomes; RI = Run-in; S = Screening; TID = three times a day; V = visit; W = week; WOCBP = women of childbearing potential.

Note 1: Laboratory samples required at Screening may be collected across multiple days to reduce blood volume drawn on a single day. Screening laboratory results must be received before Visit 2. If genetic results are still pending after 28 days, the screening window may be extended up to 2 additional weeks, with approval by the Medical Monitor. If a patient's screening window is extended, the patient is not required to repeat screening assessments. Laboratory assessments for all other visits should be collected prior to the first daily dose of CDCA.

Note 2: Patients will be contacted (via telephone call) 30 (±7) days after the last dose of study medication to ascertain patient safety (see Section 6.6.2 and Section 6.8 for details).

[a] Assessments for Visits 3, 4, 11, 12, and 13 may be conducted by a home health vendor at an alternative site than the Investigator's site.

[b] PK blood draws will be collected at Visit 5. PK samples will be collected predose and at 0.5, 1, 2, 3, 6, and 8 hours postdose. Patients will need to hold the first dose of study medication for on-site administration to facilitate the correct timing of the blood draw for the PK analysis. PK samples will only be obtained from patients who are on 750 mg (250 mg TID). A standardized low-fat meal will be provided 45-60 minutes prior to the first study medication dose and should be completed within 30 minutes of starting the meal. Predose blood sample for plasma PK will be drawn within 15-30 minutes post completion of the meal. After the first study medication dose, the 0.5 hours post dose draw has a ±5-minute window. The 1, 2, and 3-hour post dose draws have a ±10-minute window. An optional small snack may be offered and completed within 30 minutes of the post 3-hour timepoint. The 6-hour post dose draw has a ±15-minute window. A second standardized low-fat meal is provided 45 to 60 minutes prior to the 8-hour timepoint and the meal will be completed within 30 minutes of starting the meal. The 8-hour PK draw will be taken 15-30 minutes after the completion of the meal.

[c] If open-label rescue medication criteria are triggered prior to Visits 10 or 18, all assessments must be conducted prior to administration of open-label treatment.

[d] If prior genetic confirmation of CTX is not available, blood will be drawn at Visit 1 for gene sequencing. No patient with negative genetic sequencing will be randomized.

[e] CTX medical history should include a complete list of CTX-related symptoms present at the time of Screening.

[f] Serum pregnancy tests will be performed at Screening for WOCBP. Urine pregnancy tests will be performed at all other visits. A positive urine pregnancy test will be confirmed by a serum test. All WOCBP must have a negative pregnancy test (urine, with positive results confirmed by serum) at every visit.

[g] The CGI-C needs to be completed at this visit if a patient leaves the study early during DB1 or DB2.

[h] Ophthalmology exam includes BCVA and cataracts. The ophthalmology exam has a window of ±1 day to accommodate being conducted by a separate specialist.

[i] At Screening, the physical examination is a full physical examination; at subsequent visits, the physical examination can be an abbreviated exam. Height will be measured at Screening, baseline (Visit 6/Day 1), and EOS for adult cohort patients. Physical examinations will include assessment of the following body systems: abdomen; cardiovascular; ear, nose, and throat; eyes; hair and skin; lymph nodes; mental status; musculoskeletal; neurological; and respiratory.

[j] The Investigator will assess if a patient requires OL rescue medication at each visit of a DB period including Visits 10 and 18 when OL medication is dispensed per protocol. If OL rescue medication is required, the Investigator must complete and submit a Clinical Progression Requiring Rescue Form (Appendix 5). If OL rescue medication is not required, the rescue form must be submitted at the completion of each DB period (ie, Visit 10 and Visit 18).

[k] Vital signs will always be measured prior to having blood drawn for laboratory evaluations.

[l] All adult cohort patients (regardless of their history of CDCA use) and/or their parent/legal guardian will be asked to complete the daily and per-event PRO diaries for bowel function and seizures during the 7 days prior to Visit 4 (OL1), Visit 6 (DB1), Visit 14 (DB2), and throughout the duration of each double-blind period. Reminders regarding the completion of the PRO diary will be sent to patients and/or patient's parent/legal guardian at times specified in the Study Manual. Diaries will be reviewed for compliance at each visit that it is required. If the review of the PRO diary indicates that the patient or the patient's parent/legal guardian is not sufficiently compliant with completion of the diary as described in the Study Manual, the patient or the patient's parent/legal guardian will be retrained on how to complete the diary.

[m] Distribution and training on use of diary only.

[n] Patients who are treatment-naïve or have been treated with CDCA for ≤2 months prior to Screening will also complete the daily and per-event PRO diaries for bowel function and seizures during the 7 days prior to Visit 3 and Visit 5 (OL1). Diaries will also be reviewed for compliance at these visits.

[o] First morning void on 3 mornings within 5 days prior to the visit will be collected and brought to the site visit. For the Screening visit, the patient may bring these urine samples to the site after the Screening visit and before the first day of the OL1 period.

[p] Collected only from patients on anticoagulants at all visits, except Visits 7, 9, 15, and 17.

[q] A list of clinical laboratory assessments is provided in Appendix 3.

[r] Blood draw for thyroid stimulating hormone will only be collected at Screening.
[s] Fasting blood draws for the assessment of lipid parameters will be collected at Visits 2, 6, 10 (EOT1), 14, and 18 (EOT2/EOS/ET).
[t] It is anticipated that some patients will have a worsening of the underlying condition of the disease, such as increasing diarrhea. Any such conditions considered by the Investigator to be worsening of the underlying condition should be reported as AEs. Investigators should also report such AEs as part of the clinical rescue assessment as appropriate (Section 12.4).
[u] EEG has a window of ±1 day to accommodate being conducted by a separate specialist.
[v] Patients will need to hold the first dose of study medication for on-site administration.
[w] In OL1 and OL2, open-label study medication is dispensed at Visit 4 and Visit 12, respectively.

APPENDIX 2. SCHEDULE OF ASSESSMENTS FOR PEDIATRIC COHORT

	V1	V2	V3[a], 5[a], 7[a], 9[a]	V4, 6, 8	V10	V12[a], 14[a], 16[a]	V11, 13, 15, 17	V18
		Titration Period				Treatment Period		
Week	**Screening**	**Titration Start**	**Titration W1, 3, 5, 7**	**Titration W2, 4, 6**	**Treatment Start Day 1[b]**	**W4, 8, 12**	**W2, 6, 10, 14**	**W16 EOT EOS ET**
Visit Window	≤28 days prior to Titration start	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day
Informed Consent/Assent	X							
Inclusion/Exclusion	X							
Genetic Testing for CTX[c]	X							
Serologic test for Hep B, Hep C, HIV	X							
Medical History	X							
CTX Medical History[d]	X							
Prior Medication	X							
Demographics	X							
Pregnancy Test[e]	X	X	X	X	X	X		X
Concomitant Medication	X	X	X	X	X	X		X
Health Status During Prior Week		X		X	X	X		X
CGI-S					X			
CGI-C						X		X[f]
Motor Developmental Milestones[g]		X						X
Ophthalmology Exam[h]		X						X
Physical Examination (including body weight)[i]	X	X		X	X	X		X
Vital Signs[j]	X	X	X	X	X	X		X

	V1	V2	V3[a], 5[a], 7[a], 9[a]	V4, 6, 8	V10	V12[a], 14[a], 16[a]	V11, 13, 15, 17	V18
		Titration Period				Treatment Period		
Week	**Screening**	**Titration Start**	**Titration W1, 3, 5, 7**	**Titration W2, 4, 6**	**Treatment Start Day 1[b]**	**W4, 8, 12**	**W2, 6, 10, 14**	**W16 EOT EOS ET**
Visit Window	≤28 days prior to Titration start	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day
Patient Diary[k]	X[l]			X	X	X		X
Urine 23S-pentol (3 first morning voids)[m]	X	X	X	X	X	X		X
Urinalysis Assessments	X	X	X	X	X	X		X
Blood draw for Cholestanol, 7αC4, 7α12αC4, and Plasma Bile Alcohol	X	X		X	X	X		X
Laboratory Assessments (including LFTs)[n, o]	X[p]	X		X	X	X		X
AST, ALT, Bilirubin			X					
Pharmacokinetic Blood Sample Collection[q]		X[q]		X[q]		X[q]		
Adverse Events[r]			Collected continuously					
EEG[s]		X			X	X[t]		X
12-Lead ECG	X			X				X
Study Medication Administration[u]		X	X	X	X	X	X	
Dispense Study Medication		X		X	X	X	X[v]	
Medication Accountability			X	X	X	X		X

Abbreviations: AST = aspartate aminotransferase; ALT = alanine aminotransferase; BCVA = best-corrected visual acuity; CDCA = chenodeoxycholic acid; CGI-C = Clinician Global Impression of Change; CGI-S = Clinician Global Impression of Severity; CTX = cerebrotendinous xanthomatosis; ECG = electrocardiogram; EEG = electroencephalogram; EOS = end of study; EOT = end of treatment; ET = Early Termination; Hep B = hepatitis B virus surface antigen; Hep C = hepatitis C; HIV = human immunodeficiency virus; LFT = liver function test; PK = pharmacokinetics; PRO = Patient-reported outcomes; S = Screening; V = visit; W = week; WOCBP = women of childbearing potential.

Note 1: Laboratory samples required at Screening may be collected across multiple days to reduce blood volume drawn on a single day. Screening laboratory results must be received before Titration Period W1. If genetic results are still pending after 28 days, the screening window may be extended up to 2 additional weeks, with approval by the Medical Monitor. If a patient's screening window is extended, the patient is not required to repeat screening assessments. Laboratory assessments for all other visits should be collected prior to the first daily dose of CDCA.
Note 2: Patients will be contacted (via telephone call) 30 (±7) days after the last dose of study medication to ascertain patient safety (see Section 6.6.2 and Section 6.8 for details).

[a] Assessments for Visits 3, 5, 7, 9, 12, 14, and 16 may be conducted by a home health vendor at an alternative site than the Investigator's site. However, the EEG assessment at Visit 14 must be conducted on site.
[b] Patients will need to hold the first dose of study medication for on-site administration.
[c] If prior genetic confirmation of CTX is not available, blood will be drawn at Visit 1 for gene sequencing. No patient with negative genetic sequencing will be enrolled.
[d] CTX medical history should include a complete list of CTX-related symptoms present at the time of Screening.
[e] Serum pregnancy tests will be performed at Screening for WOCBP. Urine pregnancy tests will be performed at all other visits. A positive urine pregnancy test will be confirmed by a serum test. All WOCBP must have a negative pregnancy test (urine, with positive results confirmed by serum) at every visit.
[f] The CGI-C needs to be completed at this visit if a patient leaves the study early during the treatment period.
[g] Note that after the baseline assessment at Titration start, there will be a trigger question to ask if there have been any changes to previous milestones assessed (either new milestone met or a regression).
[h] Ophthalmology exam includes BCVA and cataracts. The ophthalmology exam has a window of ±1 day to accommodate being conducted by a separate specialist.
[i] At Screening, the physical examination is a full physical examination; at subsequent visits, the physical examination can be an abbreviated exam. Height, weight, and head circumference will be measured at all visits for pediatric cohort patients to be able to determine Failure to Thrive. Physical examinations will include assessment of the following body systems: abdomen; cardiovascular; ear, nose, and throat; eyes; hair and skin; lymph nodes; mental status; musculoskeletal; neurological; and respiratory.
[j] Vital signs will always be measured prior to having blood drawn for laboratory evaluations.
[k] All pediatric cohort patients (regardless of their history of CDCA use) and/or their parent/legal guardian will be asked to complete the daily and per-event PRO diaries for bowel function and seizures during the 7 days prior to Visits 4, 10, 14, and 18. Reminders regarding the completion of the PRO diary will be sent to patients and/or patient's parent/legal guardian at times specified in the Study Manual. Diaries will be reviewed for compliance at Visits 4, 10, 14, and 18. If the review of the PRO diary indicates that the patient or the patient's parent/legal guardian is not sufficiently compliant with completion of the diary as described in the Study Manual, the patient or the patient's parent/legal guardian will be retrained on how to complete the diary.
[l] Distribution and training on diary only.
[m] First morning void on 3 mornings within 5 days prior to the visit will be collected and brought to the site visit. For the Screening visit, the patient may bring these urine samples to the site after the Screening visit and before the first day of the Titration period.
[n] Laboratory tests will be conducted at a central laboratory. During the titration period of the pediatric cohort, testing at a local laboratory is permitted to facilitate titration review if there are delays to central laboratory reporting (eg, lost or hemolyzed blood sample). A list of clinical laboratory assessments is provided in Appendix 3.
[o] Fasting blood draws for the assessment of lipid parameters will be collected at Visits 2 (Titration Start), 8 (Titration Week 6), 12 (Week 4), 16 (Week 12), 18 (Week 16/EOT/EOS/ET).
[p] Blood draw for thyroid stimulating hormone will only be collected at Screening.

[q] Blood sample for plasma PK will be drawn within 1 hour prior to study medication administration. PK samples will be collected predose (t0), and at 1 and 3 hours postdose at Visit 2 (Titration Start). PK samples will be collected at Visits 4, 6, or 8 only if there is a dose change. Additional PK samples will be collected at Visit 12 if a steady dose has been maintained for ≥1 month prior to the visit. If a steady dose was not maintained for ≥1 month prior to Visit 12, PK sample collection may be delayed to Visit 14. If a steady dose was not maintained for ≥1 month prior to Visit 14, PK sample collection may be delayed and will occur at Visit 16. Patients will need to hold first daily dose of study medication for on-site administration to facilitate the correct timing of the blood draw for the PK analysis. A standardized low-fat meal will be provided 45-60 minutes prior to dosing and should be completed within 30 minutes of starting the meal. Predose blood sample for plasma PK will be drawn within 15-30 minutes post completion of the meal. After the first study medication dose, the 0.5 hours post dose draw has a ±5-minute window. The 1, 2, and 3 hour post dose draws have a ±10-minute window.

[r] Adverse event information should be collected by Investigators throughout the study, regardless of where the visit takes place (ie, on-site or homecare).

[s] EEG has a window of ±1 day to accommodate being conducted by a separate specialist.

[t] EEG will be performed only at Visit 14.

[u] Treatment Day 1 occurs one week after Visit 9 (Titration Week 7).

[v] Study medication may be shipped directly to the patient or made available for dispensation at the site.

APPENDIX 3. CLINICAL LABORATORY ASSESSMENTS

Clinical Chemistry	**Hematology**	**Routine Urinalysis**
Sodium Potassium Chloride Bicarbonate Total protein Albumin Calcium Phosphate Glucose Blood Urea Nitrogen Creatinine Total bilirubin Direct bilirubin Alanine aminotransferase Aspartate aminotransferase Gamma-glutamyl transferase Lactate dehydrogenase Alkaline phosphatase Creatine kinase **Thyroid stimulating hormone** **Serologic Testing** Hep B Hep C HIV **Biomarkers** Cholestanol 7αC4 7α12αC4 Urine 23S-pentol Plasma tetrol-glucuronide (plasma bile alcohol)	Red blood cell count Hemoglobin Hematocrit MCV, MCH, MCHC RBC distribution width Platelet count White blood cell count WBC differential (% and absolute) • Neutrophils • Eosinophils • Basophils • Lymphocytes • Monocytes	Color Appearance pH Specific gravity Protein Glucose Ketones Bilirubin Blood Urobilinogen Nitrates Leukocyte esterase Microscopic examination (performed if blood, protein, or leukocyte esterase is abnormal in urine) For WOCBP, pregnancy test (confirmed with serum test if positive)
	Coagulation (adult cohort only) Prothrombin time INR	**Lipid Profile** Total cholesterol Triglycerides HDL LDL

Abbreviations: 7αC4 = 7 alpha hydroxy 4 cholesten-3 one; 7α12αC4 = 7-alpha,12-alpha-dihydroxy-4-cholesten-3-one; HDL = high-density lipoprotein; Hep = hepatitis; HIV = Human immunodeficiency virus; INR = international normalized ratio; LDL = low density lipoprotein; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; WBC = white blood cell; WOCBP = women of childbearing potential.

APPENDIX 4. PATIENT/CAREGIVER ASSESSMENT OF DISEASE-RELATED SYMPTOMS OR MANIFESTATIONS

For consistency, please enter data as close to the end of each event (ie, bowel movement and seizure) or before bedtime for symptoms or manifestations collected daily. The actual questions will reflect who fills out the questionnaire, either the patient or the caregiver.

Are you the Patient (___) or the Caregiver (___)?

Symptoms

1. Collected for each bowel movement or at the end of the day if no bowel movement occurred during the day:
   Did you/the patient in your care have a **bowel movement (BM)**? Yes [ ] No [ ]
   If YES, please fill out the BSFS reflecting the most recent bowel movement.
   If YES, please indicate if there was any discomfort in the stomach or rectal area during the most recent bowel movement:
   No discomfort [ ] Mild [ ] Moderate [ ] Severe [ ] Very severe [ ]
2. Collected for each seizure or at the end of the day if no seizure occurred during the day:
   Did you/the patient in your care have any **seizures**? Yes [ ] No [ ]
   If YES, please contact the study doctor and record the

   Duration of the seizure (seconds or minutes): _____

   Severity of seizure: Mild [ ] Moderate [ ] Severe [ ] Very severe [ ]
3. Collected at the end of the day:
   Did you/the patient in your care have any **tremors** today? Yes [ ] No [ ]
   If YES, please fill out the tremor questionnaire indicating the area(s) affected and the severity.

Area Affected		If YES			
		Mild	Moderate	Severe	Very Severe
Head	Yes [ ] No [ ]				
Face	Yes [ ] No [ ]				
Tongue	Yes [ ] No [ ]				
Neck	Yes [ ] No [ ]				
Arms/hands	Yes [ ] No [ ]				
Trunk	Yes [ ] No [ ]				
Legs/feet	Yes [ ] No [ ]				
Voice	Yes [ ] No [ ]				

Manifestations: Impact on Daily Activities

Rate the impact on performing daily activities today for you/the patient in your care due to difficulty.

	Did you/ patient in your care have difficulty (Yes/No)	If YES				
		No Impact	Slight impact, able to perform most typical daily tasks	Moderate impact, unable to perform some typical daily tasks	Severe impact, unable to perform most typical daily tasks	Very severe impact, unable to perform any typical daily task
Walking						
With weakness						
Speaking						
Using hands						
Seeing						
Thinking/ mentally focusing						
Sleeping						
Other (specify)						

Manifestations: Discomfort or Distress

Rate the discomfort or distress for you/the patient in your care due to

	Did you/the patient in your care have (Yes/No)	If YES				
		No discomfort or distress	Slight discomfort or distress	Moderate discomfort or distress	Severe discomfort or distress	Very severe discomfort or distress
Difficulty eating or swallowing						
Difficulty dressing						
Difficulty with hygiene						
Mood changes						
Difficulty urinating						
Headaches						
Pain other than headache						
Dystonia						
Other (specify)						

Most Bothersome Symptoms or Manifestations

Among the SYMPTOMS or MANIFESTATIONS that you/the patient in your care had today (those will be listed), indicate the three that bothered you/the patient in your care most today. Is there another symptom or symptoms that has/have not been captured?

APPENDIX 5. CLINICAL PROGRESSION REQUIRING RESCUE FORM

Clinical Progression Requiring Rescue

In the Investigator's clinical judgment, does the patient require rescue with open-label CDCA, based on the available information? Yes [ ] No [ ]

If yes, please indicate the specific reason(s) contributing to this decision.

Changes relative to the baseline (open-label period) preceding each double-blind period		**If YES**			
		Mild	**Moderate**	**Severe**	**Very Severe**
Increased frequency or worsening of diarrhea?	Yes [ ] No [ ]				
Increased frequency or severity of tremors?	Yes [ ] No [ ]				
Increased frequency or severity of seizures?	Yes [ ] No [ ]				
New or worsening neurological symptoms or manifestations?	Yes [ ] No [ ]				
Loss or deterioration in motor skills?	Yes [ ] No [ ]				
Reduction or deterioration of cognitive function?	Yes [ ] No [ ]				
Reduction or deterioration of visual acuity?	Yes [ ] No [ ]				
New or worsening EEG abnormality?	Yes [ ] No [ ]				
Demonstrated Failure to Thrive?	Yes [ ] No [ ]				
Decline in overall health status?	Yes [ ] No [ ]				

Additional Details

APPENDIX 6. SCHEDULE OF ASSESSMENTS DURING THE EXTENSION OF THE OPEN-LABEL PERIODS IN THE ADULT COHORT

Visit	OL1-A[a]	OL1-B [a, b]	OL2-A[a]	OL2-B[a] [b]
Visit #	Unscheduled	Unscheduled	Unscheduled	Unscheduled
Visit Frequency	Every 28 (±3) days from Visit 4 or last dispensation of OL study medication in the OL1 Period	14 (±2) days prior to the start of DB1	Every 28 days (±3) from Visit 12 or last dispensation of OL study medication in OL2 Period	14 (±2) days prior to the start of DB2
Pregnancy Test *(WOCBP only)*[c]	X	X	X	X
Concomitant Medications	X	X	X	X
Physical Examination (including body weight)	O	X	O	X
Vital Signs[d]	O	X	O	X
Urine 23S-pentol (3 first morning voids)[e,f]	O	X	O	X
Urinalysis Assessments[e]	X	X	X	X
Blood draw for Cholestanol, 7αC4, 7α12αC4, and Plasma Bile Alcohol[e]	O	X	O	X
Laboratory Assessments (including coagulation [INR and PT] and LFTs)[e,g,h]	X	X	X	X
Adverse Events[i]	X	X	X	X
On-Site Study Medication Administration[j]	X	X	X	X
Dispense OL Study Medication	X		X	
Medication Accountability	X	X	X	X

Abbreviations: CDCA = chenodeoxycholic acid; DB1 = double-blind period 1; DB2 = double-blind period 2; INR = international normalized ratio; LFT = liver function test; O = optional assessment; OL = open label; OL1 = open-label period 1; OL2 = open-label period 2; PT = prothrombin time; WOCBP = women of childbearing potential; X = required assessment.

[a] Assessments may be conducted by a home health vendor at an alternative site than the Investigator's site.

[b] OL1-B and OL2-B visits are required if a patient completes ≥2 OL1-A or OL2-A visits, respectively.

[c] Urine pregnancy tests will be performed for each WOCBP at all visits in the extension period. A positive urine pregnancy test will be confirmed by a serum test. All WOCBP must have a negative pregnancy test (urine, with positive results confirmed by serum) at every visit.

[d] Vital signs will always be measured prior to having blood drawn for laboratory evaluations.

[e] Laboratory assessments should be collected prior to the first daily dose of CDCA.

[f] First morning void on 3 mornings within 5 days prior to the visit will be collected and brought to the site visit.

[g] Collected only from patients on anticoagulants.

[h] A list of clinical laboratory assessments is provided in Appendix 3.

[i] It is anticipated that some patients will have a worsening of the underlying condition of the disease, such as increasing diarrhea. Any such conditions considered by the Investigator to be worsening of the underlying condition should be reported as adverse events. Investigators should also report such adverse events as part of the clinical rescue assessment as appropriate (Section 12.4).

[j] Patients will need to hold the first dose of study medication for on-site administration.

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Approval Task	[redacted] 04-Apr-2023 21:24:02 GMT+0000

Approval Task	[redacted]nce 05-Apr-2023 00:06:11 GMT+0000

Approval Task	[redacted] 05-Apr-2023 15:28:22 GMT+0000

Approval Task	[redacted] 07-Apr-2023 03:32:28 GMT+0000

Approval Task	Ulysses Diva Biostatistics 07-Apr-2023 04:17:00 GMT+0000

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