CLINICAL STUDY PROTOCOL

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A Phase 1/2, Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) Serotype 8 (AAV8)-Mediated Gene Transfer of Human Ornithine Transcarbamylase (OTC) in Adults with Late-Onset OTC Deficiency

Protocol Number: 301OTC01

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CONFIDENTIAL

The study will be conducted according to the International Council for Harmonisation harmonised tripartite guideline E6(R2): Good Clinical Practice.

Protocol Approval

Study Title	A Phase 1/2, Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) Serotype 8 (AAV8)-Mediated Gene Transfer of Human Ornithine Transcarbamylase (OTC) in Adults with Late-Onset OTC Deficiency
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Protocol accepted and approved by:

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Protocol Synopsis

Protocol Number:	301OTC01
Title:	A Phase 1/2, Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) Serotype 8 (AAV8)-Mediated Gene Transfer of Human Ornithine Transcarbamylase (OTC) in Adults with Late-Onset OTC Deficiency
Sponsor:	Ultragenyx Pharmaceutical Inc. 840 Memorial Drive Cambridge, MA 02139
Study Phase:	Phase 1/2
Sample Size:	The study is anticipated to enroll up to 18 subjects
Study Sites:	Approximately 20 global study sites
Indication:	OTC deficiency
Primary Objective:	• To determine the safety of single intravenous (IV) doses of DTX301 in adults with late-onset OTC deficiency.
Secondary Objectives:	• To establish a dose of DTX301 that has a meaningful increase in the rate of ureagenesis to allow further clinical development. • To evaluate the efficacy of single IV doses of DTX301 in adults with late-onset OTC deficiency, in the setting of tapering or discontinuing ammonia scavenger medications.
Exploratory Objectives:	• To assess the impact of DTX301, by dose, on the number of hyperammonemic crises during the study. • To evaluate the effect of DTX301, by dose, on urinary orotic acid levels. • To evaluate the effect of DTX301, by dose, on plasma glutamine and glutamate levels. • To assess the impact of DTX301, by dose, on the subject's neuropsychological functioning. • To assess the impact of DTX301, by dose, on the subject's quality of life (QoL). • To assess the impact of DTX301, by dose, on the use of ammonia scavengers. • To assess the impact of DTX301, by dose, on dietary protein restriction. • To describe the immune response to AAV8 capsid proteins after IV administration of DTX301. • To describe the immune response to OTC after IV administration of DTX301.
Primary Endpoints:	• The incidence of adverse events (AEs), treatment-emergent adverse events (TEAEs), and serious adverse events (SAEs) for each dosing cohort, assessed by severity and relationship to study product.

Secondary Endpoints:	• The change from baseline in the rate of ureagenesis (as measured by the generation of [^{13}C]urea over 4 hours) as determined by gas chromatography mass spectrometry over time to 52 weeks after the IV administration of DTX301. • The change from baseline (Day 0) in plasma ammonia area under the curve from time zero to 24 hours (AUC_{0-24}) over time to 52 weeks after IV administration of DTX301 in the setting of tapering or discontinuing ammonia scavenger medications.
Exploratory Endpoints:	• The number of hyperammonemic crises observed for each dose over time to 52 weeks after IV administration of DTX301. • The change from baseline in urinary orotic acid excretion over time to 52 weeks after IV administration of DTX301. • The change from baseline in serum glutamine and glutamate over time to 52 weeks after IV administration of DTX301. • Changes in responses to the [redacted] Working Memory Index (WMI), will be summarized by dose level of DTX301. • Responses to the Patient-Reported Outcomes Measurement Information System (PROMIS®) questionnaire, summarized by dose level of DTX301 over time to Week 52. • Use of ammonia scavengers, summarized by dose level of DTX301. • Change in dietary protein intake, summarized by dose level of DTX301. • The development of neutralizing antibodies to AAV8 (as determined by a cell-based assay), summarized by time point and dose level of DTX301. • The development of anti-AAV8 binding antibodies (as determined by enzyme-linked immunosorbent assay [ELISA]), summarized by time point and dose level of DTX301. • The development of anti-OTC antibodies (as determined by ELISA), summarized by time point and dose level of DTX301.
Subject Population:	**Inclusion Criteria** Each subject must meet all of the following criteria at Screening to be enrolled in this study: 1. Willing and able to provide written informed consent. 2. Males and females ≥ 18 years of age with documented diagnosis of late-onset (defined as first manifestation of signs and symptoms at > 30 days of age) OTC deficiency, confirmed via enzymatic, biochemical, or molecular testing. This may include identification of a pathogenic mutation, pedigree analysis, liver OTC activity that is < 20% of normal activity, or elevated urinary orotate (> 20 µmol/mmol creatinine) after an allopurinol challenge test. 3. Documented history of ≥ 1 symptomatic hyperammonemia event with

	ammonia ≥ 100 µmol/L. 4. Subject's OTC deficiency is stable as evidenced by either a) no clinical symptoms of hyperammonemia OR b) plasma ammonia level < 100 µmol/L within the 4-week period preceding the Screening visit. 5. Subject's plasma ammonia level on Day 1 (predose) is < 100 µmol/L, for patients who historically maintain normal ammonia levels, and the subject is clinically stable; OR subject's plasma ammonia level on Day 1 (predose) is < 200 µmol/L, for patients who historically are not able to fully control ammonia levels with baseline management, and the subject is clinically stable. If the Day 1 (predose) ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. 6. On ongoing daily stable dose of ammonia scavenger therapy for ≥ 4 weeks. 7. No known allergic reaction to any component of DTX301. 8. Willing and able to comply with study procedures and requirements, including periodic inpatient hospitalizations, frequent blood draws, and urine collections over a 24-hour period. 9. Hematology and coagulation panel results are within the normal range or, if outside the normal range, deemed not clinically significant in the opinion of the investigator. 10. Males and all females of childbearing potential must be willing to use effective contraception at the time of administration of gene transfer and for the 52 weeks following administration of DTX301 to prevent the potential transmission of the AAV vector. For male subjects, appropriate contraceptive methods include the use of a condom with spermicide. For female subjects, appropriate contraceptive methods include the use of a condom with spermicide plus at least 1 of the following: a. Oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device; b. Use of a diaphragm or cervical/vault cap; c. Previous female sterilization (surgical bilateral oophorectomy [with or without hysterectomy] or tubal ligation) at least 6 weeks prior to DTX301 administration. In case of an oophorectomy alone, the reproductive status of the subject must have been confirmed by follow-up hormone level assessment. **NOTE:** Abstinence is an acceptable form of birth control; however, appropriate contraception must be used if the subject becomes sexually active. Abstinence is defined as sexual inactivity consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea methods are not acceptable methods of contraception. A condom with spermicide is required to be used by all sexually active vasectomized males

	in the study in order to prevent potential transmission of the vector via seminal fluid. **NOTE:** Females of childbearing potential are defined as all females physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception for the duration of the study. Females are considered post-menopausal and not of childbearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (eg, age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least 6 weeks prior to enrollment. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of childbearing potential. **Exclusion Criteria:** Subjects meeting any of the following criteria at Screening will be excluded from the study: 1. At Screening or Baseline (Day 0), plasma ammonia level ≥ 100 µmol/L for patients who historically maintain normal ammonia levels; OR plasma ammonia level ≥ 200 µmol/L for patients who historically are not able to fully control ammonia levels with baseline management; OR signs and symptoms of hyperammonemia, with documented elevated ammonia level, during the 4-week period preceding Day 0. If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. 2. Liver transplant, including hepatocyte cell therapy/transplant. 3. History of liver disease as evidenced by any of the following: portal hypertension, ascites, splenomegaly, esophageal varices, hepatic encephalopathy, or a liver biopsy with evidence of stage 3 fibrosis. 4. Significant hepatic inflammation or cirrhosis as evidenced by imaging or any of the following laboratory abnormalities: alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > the upper limit of normal (ULN), total bilirubin > 1.5 × ULN, alkaline phosphatase > 2.5 × ULN. 5. Serum creatinine > 2.0 mg/dL. 6. Evidence of active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, documented by current use of antiviral therapy for HBV or HCV or by hepatitis B surface antigen (HBsAg) or HCV RNA positivity. **NOTE**: Subjects with a history of HCV infection must have documentation of 2 negative viral assays by polymerase chain reaction (PCR), collected at least 6 months apart, to be considered negative for HCV. Subjects with a history of HCV infection who test positive for HCV RNA at Screening can be rescreened once, after they have been treated and have documentation of at least 2 negative samples collected at least 6 months apart. 7. History of human immunodeficiency virus (HIV) infection AND any of the following: CD4+ cell count < 350 cells/mm^3, change in antiretroviral

	therapy regimen within 6 months prior to Day 0, or plasma viral load > 200 copies/mL, documented on 2 separate occasions, as measured by PCR. 8. Active infection (viral or bacterial). 9. Anti-AAV8 neutralizing antibody titer ≥ 1:5. 10. Participation (current or previous) in another gene transfer study. 11. Participation in another investigational medicine study within 3 months of Screening. 12. History of a malignancy for which the subject has received treatment in the past 2 years except for prostate cancer treated with watchful waiting or surgically removed non-melanoma skin cancer. 13. Has a positive serum pregnancy test at Screening (females of childbearing potential only), a positive urine pregnancy test at Baseline (Day 0; females of childbearing potential only), or is nursing. 14. Has any other significant medical condition that the investigator feels would be a risk to the subject or would impede the study.
Study Design:	This is a Phase 1/2, open-label, single arm, multicenter, safety and dose-finding study of DTX301 in adults with late-onset OTC deficiency. The primary objective of the study is to determine the safety of single IV doses of DTX301. A key element of the study is the identification of the optimal biological dose (OBD) of DTX301. The secondary objectives of the study are the assessment of rate of ureagenesis, reflecting the direct *in vivo* efficiency of the urea cycle and the assessment of plasma ammonia (AUC_{0-24}), reflecting clinical metabolic control. The target for DTX301 is to achieve a rate for ureagenesis of approximately 300 µmol/kg/hr (±10%), which is the approximate rate of ureagenesis in healthy adults. Eligible subjects will receive a single IV infusion of DTX301. Three subjects will be enrolled in Cohort 1 and a minimum of 2 to 3 subjects will be enrolled in each subsequent cohort. Dose escalation will be conducted according to a model that uses the collected data to predict the safety profile of the dose in order to determine the OBD. There will be a minimum of 12 weeks between the dosing of the last subject in one dosing cohort and the first subject in the next dosing cohort followed by data monitoring committee (DMC) review of a dosing cohort up to Cohort 3. Dosing of additional subjects as expansion of Cohort 3, and initiation of Cohort 4 (Dosing Process Optimization) may occur in parallel, following the DMC review of a minimum of 12 weeks of data from the initial 3 subjects in Cohort 3. A continual reassessment method (CRM) will be used for dose finding to discover the OBD of DTX301. Cohort 4 is intended to dose at OBD. Subjects will be followed for 52 weeks after dosing. After completion of this study, subjects will be asked to enroll in a 4-year extension study to evaluate the long-term (a total of 5 years after dosing) safety and efficacy of DTX301.
Study Methodology:	**Screening Period** After a subject has provided written informed consent, the investigator or other

	qualified study personnel will determine if the subject is eligible for the study. This will be accomplished by reviewing the inclusion and exclusion criteria and completing all of the screening assessments. Screening assessments may be performed on more than 1 day; all assessments must be completed and results available and reviewed prior to Day 0. Screening Assessments: Demographic data; OTC and general medical history; prior medications, therapies, and procedures; use of ammonia scavengers; dietary protein restrictions; vital sign measurements; height and weight will be recorded. A 12-lead electrocardiogram (ECG) and a complete physical examination will be performed. Blood samples will be collected for HBV, HCV, and HIV status; serum pregnancy test (females of childbearing potential only); AAV8 neutralizing antibody testing; AAV8 binding antibody immunoglobulin G (IgG) testing, and clinical laboratory assessments (clinical chemistry, plasma ammonia [STAT sample], hematology, urinalysis, and coagulation panel). Adverse events and SAEs will be monitored after the subject has provided written informed consent. Subjects will be administered [1-^{13}C]sodium acetate orally. Blood samples for determination of ureagenesis will be collected, via an indwelling catheter, before dosing (time 0) and at approximately 0.5, 1, 1.5, 2, 3, and 4 hours after dosing with [1-^{13}C]sodium acetate. During Screening, assessment of rate of ureagenesis may be repeated if discrepant with subject's clinical status and severity. Subjects participating in Cohort 4 (Dosing Process Optimization) will start the prophylactic corticosteroid regimen at least 5 days prior to dosing with DTX301. The subject must be assessed as clinically and metabolically stable, and intercurrent illnesses (eg, viral infection) or concomitant medications known to affects transaminases, be excluded. At any time after initiation of prophylactic corticosteroid regimen, additional assessments of plasma ammonia levels, amino acid profiles or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator and as clinically indicated. **Day 0 – Baseline Measurements** Subjects will be admitted on Day 0 for the baseline determination of the following: plasma ammonia (AUC_{0-24}) and urinary orotic acid levels over 24 hours. Prior to the determination of plasma ammonia AUC_{0-24}, blood samples will be collected for OTC genotyping, AAV8 neutralizing antibody testing, AAV8 binding antibody IgG testing, anti-OTC antibody testing, amino acid panel, and clinical laboratory assessments (clinical chemistry, plasma ammonia [STAT sample], hematology, urinalysis, and coagulation panel). Saliva, urine, and stool samples will be collected to provide a baseline for assessment of viral shedding. A urine pregnancy test will be performed on all female subjects of childbearing potential. An ECG and a targeted physical examination will be performed. Vital sign measurements and weight will be recorded. Adverse events and SAEs will be monitored; concomitant medications, therapies, and procedures, use of ammonia scavengers and dietary protein restrictions will be recorded.

	Subjects participating in Cohort 4 (Dosing Process Optimization) will start the prophylactic corticosteroid regimen at least 5 days prior to dosing with DTX301. The subject will complete the PROMIS questionnaire to evaluate QoL prior to the neuropsychological tests. The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable in order to perform the neuropsychological tests. The study site may use the time 0 (T 0) plasma ammonia sample (from AUC_{0-24} plasma ammonia) analyzed by the local laboratory (STAT sample) to determine the subject's plasma ammonia level. The result must be available prior to initiating the tests. If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. If the subject is deemed clinically unstable, the neuropsychological tests will be held. Subjects, in a nonfasted state, will complete the following neuropsychological tests: **IQ:** [redacted] **Motor Function**: [redacted] **Executive Function**: [redacted] **Memory**: [redacted] Blood samples will be collected for the determination of plasma ammonia (AUC_{0-24}) at time 0 and at approximately 2, 4, 8, 12, 16, 20, and 24 hours. Urine will be collected for the determination of orotic acid at time 0 and at approximately 6, 12, 18, and 24 hours (relative to the start of plasma ammonia determination). Urine orotic acid concentration will be standardized to urine creatinine concentration. **Day 1 – Determination of Plasma Ammonia, Ureagenesis, and DTX301 Infusion** The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable in order to receive [1-^{13}C]sodium acetate and DTX301. The 24-hour time point (T 24) from the local laboratory (STAT sample) for (AUC_{0-24}) of plasma ammonia can be used and the result available prior to administering [1-^{13}C]sodium acetate. If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. If the subject is deemed clinically unstable, administration of [1-^{13}C]sodium acetate will be held. Subjects will be administered [1-^{13}C]sodium acetate orally (morning of Day 1). Blood samples for determination of ureagenesis will be collected, via an indwelling catheter, before dosing (time 0) and at approximately 0.5, 1, 1.5, 2, 3, and 4 hours after dosing with [1-^{13}C]sodium acetate. Prior to the preparation of DTX301, a blood sample will be collected for clinical chemistry (including liver function tests) and vital sign measurements will be recorded. Adverse events and SAEs will be monitored; concomitant medications, therapies, and procedures, use of ammonia scavengers and dietary protein

	restrictions will be recorded. Prior to the start of DTX301 infusion, the study site must confirm that the subject's plasma ammonia level on Day 1 (predose) is < 100 µmol/L for patients who historically maintain normal ammonia levels, and the subject is clinically stable; OR subject's plasma ammonia level on Day 1 (predose) is < 200 µmol/L, for patients who historically are not able to fully control ammonia levels with baseline management, and the subject is clinically stable. If the Day 1 (predose) ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. NOTE: If the subject is deemed clinically unstable, dosing will be held, and the subject can be rescreened once the subject is determined to be clinically stable. The 24-hour time point (time 24) from local laboratory (STAT sample) for (AUC_{0-24}) of plasma ammonia can serve as the DTX301 predose plasma ammonia result, if drawn within 12 hours of dosing with DTX301. If not, a new plasma ammonia (STAT sample) is to be collected prior to dosing with DTX301. Subjects will receive a single IV infusion of DTX301. The start of DTX301 infusion should be after all samples and procedures specified for Day 0 and Day 1 (predose) have been completed. Subjects will be discharged after a 24-hour observation period. After dosing with DTX301, a blood sample will be collected for clinical laboratory assessments (clinical chemistry [including liver function tests]) at approximately 0.5, 4, 8, and 22 hours after the start of infusion. A second sample for liver function tests only will be collected and sent to the local laboratory (STAT sample). Vital sign measurements will be recorded at 5 minutes after the start of infusion, and at approximately 0.5, 1, 2, 4, 6, 8, and 22 hours after the start of infusion. An ECG will be performed at 1 hour after the start of infusion. A single sample for plasma ammonia (STAT sample at local laboratory) will be collected and reviewed prior to discharging the subject. Subjects treated in Cohort 4 (Dosing Process Optimization) will utilize an alternative regimen of corticosteroids, aiming at prophylaxis of vector-induced hepatitis. Therefore, oral prednisone (or prednisolone) will be initiated before dosing with DTX301, sustained for 4 weeks, followed by tapering. At any time after initiation of prophylactic corticosteroid regimen, additional assessments of plasma ammonia levels, amino acid profiles or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator and as clinically indicated. **Clinic or Home Visits** Subjects will be asked to provide samples for clinical chemistry approximately every 4 days through Week 12 of the study. One sample for LFTs is collected as part of clinical chemistry and sent to the central laboratory for analysis. A second sample for LFTs only is collected and sent to the local laboratory (STAT sample). Through Week 12, one sample for spot ammonia will be collected approximately once per week and sent to the local laboratory (STAT sample). If subjects cannot visit the study site in person, subjects may have the option of being visited by clinically trained and qualified personnel (if available) approximately every 4 days at their home through Week 12; the exception being required study site visits when the subject must visit the study site in person. At any point between scheduled visits, additional unscheduled assessment of LFTs,

	plasma ammonia, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator. **Weeks 2, 4, 10, 16, 20, and 36 (Outpatient Clinic Visit)** (Table 15-1) Where available, agreed upon by the investigator and allowed by local regulation, an outpatient clinic visit may take place as home health services. **Week 20 visit is a mandatory outpatient clinic visit for all subjects** (Table 15-1)**.** **Weeks 6, 12, and 24 (Inpatient Visit)** (Table 15-1) Subjects will be discharged following completion of all assessments, including plasma ammonia (AUC_{0-24}), urinary orotic acid levels over 24 hours, and ureagenesis. Subjects will be housed for approximately 28 hours. **End of Study (Week 52)/Early Withdrawal Visit (Inpatient Visit)** A comprehensive visit will occur at Week 52 (Table 15-1). Subjects will be discharged following completion of all assessments, including plasma ammonia (AUC_{0-24}), urinary orotic acid levels over 24 hours, ureagenesis, and neuropsychological tests. Subjects will be housed for approximately 28 hours. Following completion of the Week 52 visit, subjects will be asked to enroll in a 4-year extension study to evaluate the long-term (a total of 5 years after dosing) safety and efficacy of DTX301.

Tapering of Ammonia Scavenger Therapy and Protein-Restricted Diet:	There are multiple types of ammonia scavenger medications available globally and patients with OTC deficiency typically have a personalized prescription of one or more ammonia scavenger medications to optimize the management of their disease. Adjustments to ammonia scavenger therapy may be considered following the Week 12 and Week 24 visits. Changes to baseline treatment (ie, ammonia scavenger therapy and protein-restricted diet) cannot occur until there is evidence of transgene expression reflected by continued evidence of metabolic stability with ammonia levels in the normal range or improved ammonia control if subjects have a history of ammonia levels above the upper limit of normal and supported by improvement in clinical signs and symptoms. The subject must be clinically stable and under good metabolic control before changes can be initiated or progressed. The risks of making adjustments to baseline treatment on their own, without express guidance from the site, will be reinforced with the subject at site visits. Changes to baseline treatment cannot occur while the subject is treated with corticosteroids or within a 2-week period of completing a corticosteroid taper. Modification of ammonia scavenger therapy cannot occur at the same time as changes in protein-restricted diet. Once medications or diet are adjusted or discontinued, the subject must demonstrate good metabolic control prior to adjustments in the other type of baseline treatment. Modification of baseline treatment will be individualized, dependent on the clinical judgment of the investigator, and based on review of the totality of longitudinal clinical and laboratory data for each subject, including spot plasma ammonia levels, plasma ammonia (AUC_{0-24}), subject clinical stability/asymptomatic status, neurocognitive status, and subject-reported outcomes**. Rate of ureagenesis cannot be used for decision-making in modification of ammonia scavenger therapy or protein-restricted diet and results will not be made available to the investigative sites until the end of the study.** During periods of adjustment to baseline treatment, subjects will be closely monitored. At any time after modification and/or discontinuation of ammonia scavenger therapy, reinstitution of therapy may take place based on the subject's clinical and metabolic status (elevated ammonia levels or signs and symptoms consistent with hyperammonemia), and under evaluation of the investigator.

Tapering of Ammonia Scavenger Therapy and Protein-Restricted Diet (continued):	Reinstitution of ammonia scavenger therapy should be considered if: 1. Subjects demonstrate 2 or more documented consecutive laboratory results of hyperammonemia (plasma ammonia level ≥ 100 µmol/L). NOTE: If the subject is clinically stable, spot ammonia may be repeated if discrepant with subject's clinical status and severity, OR 2. Subjects demonstrate upward trending plasma ammonia levels less than 100 µmol/L and associated with clinical signs or symptoms suggestive of worsening underlying disease, OR 3. Subjects experience a time of increased metabolic demand, such as intercurrent viral infection, treatment with steroids, hyperammonemia, or hyperammonemic crisis. Learnings from this study will allow for the development of a more standardized protocol for tapering ammonia scavenger medications in future studies of DTX301.
Occurrence of Hyperammonemia and Hyperammonemia Crisis (HAC)	Consistent with the Urea Cycle Disorders Consortium (UCDC), we have defined hyperammonemic crisis (HAC) in this study as an episode of signs and symptoms associated with hyperammonemia (such as frequent vomiting, nausea, headache, lethargy, irritability, combativeness, and somnolence), with documented elevated ammonia levels (≥ 100 µmol/L) and requiring medical intervention [Kent and Holt 2017; Longo and Holt 2017; Diaz 2019]. On-study management of hyperammonemia plasma level or hyperammonemic crises may include, but is not limited to, intravenous hydration, discontinuation of all dietary protein intake, increase in ammonia scavenger therapy, hemodialysis in addition to treatment of underlying inciting illness or event (eg, acute infection, sepsis, bleeding, drug-related, etc.). Comprehensive, internationally accepted guidelines for management of urea cycle disorders are recommended [Häberle 2012; Häberle 2019]. Because there is a wide spectrum of presentation and severity of symptomatic hyperammonemia events, investigator judgment needs to be exercised and each subject's management individualized (Section 3.2.4).

Treatment for Vector-Induced Hepatitis:	The investigator, in conjunction with the Ultragenyx Pharmaceutical medical lead will consider starting oral steroid treatment, per protocol, for possible vector-induced hepatitis when a subject's ALT is greater than the ULN and is considered by the investigator to be related to treatment with DTX301. The subject must be assessed as clinically and metabolically stable, and intercurrent illnesses (eg, viral infection) or concomitant medications known to affect transaminases, be excluded. Repeat liver function testing for confirmation of results should be considered to inform the use of steroid treatment. If repeat testing is deemed necessary, every effort should be made to repeat the testing within 24 hours from receipt of test results indicating elevated ALT. At any point between scheduled visits, additional, unscheduled assessments for LFTs, plasma ammonia or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator. Changes to baseline treatment (ammonia scavenger medications and protein-restricted diet) cannot occur while the subject is treated with corticosteroids or within a 2-week period of completing a corticosteroid taper. Based on available evidence, it is expected that vector-induced hepatitis will be self-limiting. Therefore, oral prednisone (or prednisolone) will be used per the American Association for the Study of Liver Disease guidelines in **Cohorts 1 to 3**, with a slight modification: • Week 1: prednisone 60 mg/day • Week 2: prednisone 40 mg/day • Week 3 and Week 4: prednisone 30 mg/day • Week 5 and thereafter: Prednisone will be tapered by 5 mg/week until liver enzymes return to baseline levels. Cohort 4 (Dosing Process Optimization) will utilize an alternative regimen of corticosteroids, aiming at prophylaxis of vector-induced hepatitis. Therefore, oral prednisone (or prednisolone) will be initiated before dosing with DTX301, sustained for 4 weeks, followed by tapering as described below. Prophylactic corticosteroid regimens are in use for gene transfer therapies [Audentes 2017 Clinicaltrials.gov identifier: NCT03223194; Abeona Therapeutics 2016 ClinicalTrials.gov identifier: NCT02716246]. • At least 5 days prior to administration of DTX301: prednisone 60 mg/day • Study Day 1 (Week 1) through Week 4: prednisone 60 mg/day • Study Week 5 through Week 8, tapering every 5 days: • Prednisone 40 mg/day for 5 days, followed by prednisone 30 mg/day for 5 days, prednisone 20 mg/day for 5 days, prednisone 10 mg/day for 5 days, prednisone 5 mg/day for 5 days, and prednisone 5 mg every other day for 5 days

	At any time after initiation of prophylactic corticosteroid regimen, additional assessments of plasma ammonia levels, amino acid profiles or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator and as clinically indicated. Utilization of prednisone (or prednisolone) after dosing and after completion of the prophylactic regimen, remains available to all subjects when a subject's ALT is greater than the ULN and is considered by the investigator to be related to treatment with DTX301.
Safety Stopping Criteria:	Enrollment will be stopped and the regulators will be notified if, at any time during the study, any of the following occur: • Death of a subject following administration of DTX301 • An event with an intensity of ≥ Grade 3 (according to the Common Terminology Criteria for Adverse Events), but excluding hyperammonemic crises, develops following administration of DTX301 • Occurrence of a hepatic malignancy following administration of DTX301 Abnormal clinically significant laboratory values (clinical chemistry, plasma ammonia, hematology, urinalysis, and coagulation panel) as assessed by the investigator will be considered AEs. Laboratory values that meet any of the above criteria will result in a pause in enrollment until the DMC reviews the data and provides a recommendation for subsequent study conduct. Any event that meets any of the above criteria will be reported immediately and captured as an AE/SAE in the electronic case report form (eCRF), as appropriate. Enrollment and dosing will be temporarily suspended until the situation can be assessed and risks to the subjects mitigated. If an investigator reports an AE/SAE that meets any of the study stopping criteria, the DMC will meet on an ad hoc basis to review and assess the event. If study enrollment is suspended, all subjects who have been enrolled will remain in the study and will continue to be monitored through their completion or withdrawal from the study. All AEs will be graded according to the most current version of the National Cancer Institute Common Terminology Criteria for Adverse Events.
Efficacy Assessments:	Efficacy assessments include: The rate of ureagenesis, as measured by the generation of [^{13}C]urea over 4 hours, will be determined by GCMS at Screening, on Day 1 and at 6, 12, 20, 24, and 52 weeks after IV administration of DTX301. During Screening, assessment of rate of ureagenesis may be repeated if discrepant with subject's clinical status and severity. The change from Baseline (Day 0) in AUC_{0-24} plasma ammonia at 6, 12, 24, and 52 weeks after IV administration of DTX301.

Safety Assessments:	Safety assessments will include the following: AEs, SAEs, complete and targeted physical examination findings, vital sign measurements, ECG results, clinical laboratory assessments (clinical chemistry, plasma ammonia, hematology, urinalysis, and coagulation panel), viral shedding, measurement of neutralizing antibody titer to AAV8, measurement of AAV8 binding antibodies, and measurement of anti-OTC antibodies.
Pharmacokinetic/ Pharmacodynamic Assessments:	**Ureagenesis** The conversion of the stable isotope [1-^{13}C]sodium acetate to [^{13}C]urea will be determined by GCMS. Blood samples will be collected in precooled heparinized tubes before dosing (time 0) and at approximately 0.5, 1, 1.5, 2, 3, and 4 hours after [1-^{13}C]sodium acetate is administered orally at Screening, Baseline (Day 1), and over time to Week 52 after administration of DTX301. Blood samples will be immediately centrifuged to separate the plasma, which will be stored frozen at -70°C until shipped for analysis. During Screening, assessment of rate of ureagenesis may be repeated if discrepant with subject's clinical status and severity. **Plasma Ammonia** The AUC_{0-24} of plasma ammonia will be determined at Baseline (Day 0) and over time to Week 52 after administration of DTX301. Two samples (one analyzed at the local laboratory [STAT sample], and the other analyzed at the central laboratory) will be collected at time 0 and at approximately 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours. **Orotic Acid Excretion** The excretion of orotic acid will be determined over a 24-hour period at Baseline (Day 0) and over time to Week 52 after administration of DTX301.
Estimated Study Duration:	The duration of the study is defined for each subject as the date signed written informed consent is provided through the visit at Week 52. Subjects will be in the study for approximately 56 weeks (including the screening period). After completion of this study, subjects will be asked to enroll in a 4-year extension study to evaluate the long-term (a total of 5 years after dosing) safety and efficacy of DTX301.
Study Product, Dose, and Route of Administration:	DTX301 will be administered as a single peripheral IV infusion. A continual reassessment method (CRM) will be used to determine the OBD. The following candidate doses may be evaluated to determine the OBD: • Dose 1: 2.0×10^{12} genome copies (GC)/kg • Dose 2: 6.0×10^{12} GC/kg • Dose 3: 1.0×10^{13} GC/kg Based on the results of the CRM, additional doses may be considered.
Statistical Methods:	**Determination of the Optimal Biological Dose** A CRM will be used for dose finding to discover the OBD of DTX301. The CRM uses the Bayesian method to model the probability of experiencing a

	dose-limiting toxicity (DLT) for each given dose in order to determine the next dose. A DLT is defined as any AE/SAE ≥ Grade 3 that is considered related to DTX301 by the investigator. Noninformative as well as informative prior distributions (ie, historical data) for the model parameters can be used to help with the initial modeling of the probability of experiencing a DLT. The OBD is defined as the highest dose where the predicted probability of a DLT is less than the target toxicity level. Cohort 4 is intended to dose at the OBD. Subjects may be assigned in parallel to either expansion of Cohort 3 or Cohort 4, with at least 1 male subject assigned to each. **Interim Analysis** An interim analysis may be conducted when 12-week data are available for all subjects from at least 2 cohorts. **Safety Analyses** All statistical analyses of safety outcomes will be descriptive. The incidence of AEs and TEAEs will be summarized for each dosing cohort by severity and relationship to study product. Serious AEs will be presented for each dosing cohort by relationship to study product. Summary tables will present incidence estimates and individual event rates by system organ class as well as within each system organ class. Subjects experiencing an event more than once with varying severity will be counted only once, in the maximum severity within each system organ class and preferred term. For incidence of relationship to study product, subjects will be counted only once, in the category of the strongest relationship to study product within each system organ class/preferred term.

List of Abbreviations

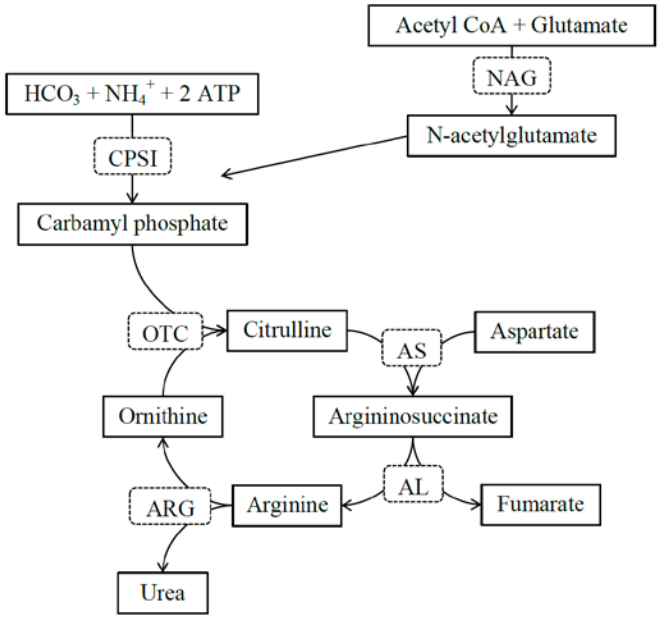
Abbreviation	Definition
AAV	adeno-associated virus
AAV2	adeno-associated virus serotype 2
AAV8	adeno-associated virus serotype 8
AE	adverse event
ALT	alanine aminotransferase
AUC_{0-24}	area under the curve from time zero to 24 hours
CFR	Code of Federal Regulations
CRA	clinical research associate
CRM	continual reassessment method
CTCAE	Common Terminology Criteria for Adverse Events
[redacted]	[redacted]
DLT	dose-limiting toxicity
DMC	data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
GC	genome copies
GCP	Good Clinical Practice
HAC	hyperammonemic crisis
HBV	hepatitis B virus
HCV	hepatitis C virus
IBC	institutional biosafety committee
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IgG	immunoglobulin G
IRB	institutional review board
IV	intravenous
MED	minimal effective dose
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation	Definition
MTD	maximum tolerated dose
NCI	National Cancer Institute
OBD	optimal biological dose
OTC	ornithine transcarbamylase
PCR	polymerase chain reaction
PROMIS	Patient-Reported Outcomes Measurement Information System
PVG	pharmacovigilance
QoL	quality of life
[redacted]	[redacted]
SAE	serious adverse event
SAP	statistical analysis plan
SUSAR	serious, unexpected, suspected adverse drug reaction
UCD	urea cycle disorder
UCDC	Urea Cycle Disorders Consortium
ULN	upper limit of normal
WAIS-IV	Wechsler Adult Intelligence Scale, Fourth Edition
WASI-II	Wechsler Abbreviated Scale of Intelligence, Second Edition
WMI	Working Memory Index

1 Introduction

The urea cycle is a series of biochemical reactions that is responsible for removing excess nitrogen, in the form of ammonia, from the body (Figure 1-1). In mammals, ammonia from the breakdown of dietary and endogenous protein is converted into urea in the liver; urea is nontoxic, water soluble, and easily excreted in the urine [Deignan 2008]. Urea cycle disorders (UCDs) result when there is a deficiency of any 1 of the 6 enzymes and 2 transporters that comprise the urea cycle: N-acetylglutamate synthase, carbamyl phosphate synthetase I, ornithine transcarbamylase (OTC), argininosuccinate synthetase, argininosuccinate lyase, arginase, citrin, or ornithine translocase [Jackson 1986]. Clinically, UCDs (which are estimated to affect approximately 1 in 30,000 individuals worldwide) are characterized by excessive levels of plasma ammonia (hyperammonemia), which is a neurotoxin that can lead to irreversible cognitive impairment, coma, and death in the absence of immediate medical attention [Batshaw 1987; Krivitzky 2009].

Figure 1-1 Overview of the Urea Cycle



Abbreviations: AL, argininosuccinate lyase; ARG, arginase; AS, argininosuccinate synthetase; ATP, adenosine triphosphate; CoA, coenzyme A; CPSI, carbamyl phosphate synthetase I; HCO_3, bicarbonate; NAG, N-acetylglutamate synthase; NH_4^+, ammonium ion; OTC, ornithine transcarbamylase.

Ornithine transcarbamylase deficiency, the most common of the UCDs, is currently estimated to occur in up to 1:62,000 live births [Lichter-Konecki 2013]. Ornithine transcarbamylase deficiency is an X-linked disorder that results from mutations in the *OTC* gene, affecting the expression or activity of the OTC protein [Caldovic 2015]. A deficiency in OTC activity prevents the normal flux of ammonia through the urea cycle and ultimately results in hyperammonemia. Ornithine transcarbamylase deficiency presents as a severe form (ie, complete OTC deficiency) in males shortly after birth (neonatal onset; ≤ 30 days of age) or later in life in both males and females (late onset; > 30 days of age) with disease that ranges from mild to severe depending on the residual activity of OTC [Tuchman 2008a].

Males with neonatal-onset OTC deficiency, defined as the onset of signs and symptoms by 30 days of age, are typically normal at birth, but become symptomatic and critically ill within 2 to 3 days of initial presentation [Lichter-Konecki 2013]. Without treatment, these patients may fall into a hyperammonemic coma and develop severe neurologic abnormalities [Krivitzky 2009].
The prognosis of a newborn that falls into a hyperammonemic coma depends on the duration, rather than the magnitude, of the elevated ammonia level [Msall 1984]. Despite optimal management, a neonate with severe OTC deficiency may only tolerate as much as 1.5 g/kg/day of dietary protein, which is the minimum amount of dietary protein needed to grow. In contrast, males with partial OTC activity or heterozygous females can present with initial symptoms at any time from infancy through adulthood and are clinically classified as late-onset patients.

Symptomatic hyperammonemic events, classified as hyperammonemic crises (HAC) are often triggered by acute stressors, most commonly concurrent infection, but can become a life-threatening event at any age [Lichter-Konecki 2013; Batshaw 2014]. For example, adults with late-onset OTC deficiency have become hyperammonemic after crush injury, following surgery, when on a high-protein diet, during the postpartum period, and when treated with chemotherapy, high-dose corticosteroids, valproate, or haloperidol [Lichter-Konecki 2013; Batshaw 2014]. Consistent with the Urea Cycle Disorders Consortium (UCDC), we have defined hyperammonemic crisis (HAC) in this study as an episode of signs and symptoms associated with hyperammonemia (such as frequent vomiting, nausea, headache, lethargy, irritability, combativeness, and somnolence), with documented elevated ammonia levels (≥ 100 µmol/L) and requiring medical intervention [Kent and Holt 2017; Longo and Holt 2017; Diaz 2019].

Successful rescue from an initial hyperammonemic crisis does not eliminate the chronic risk patients have for developing repeat episodes of hyperammonemia, which may present as lethargy, seizures, or coma. Regardless of the age of onset, neuropsychological complications for all individuals with OTC

deficiency typically include developmental delay, learning disabilities, intellectual disability, attention deficit hyperactivity disorder, and executive function deficits.

The current standard of care for OTC deficiency is to limit dietary protein intake and supplement the diet with a high-energy source, such as glucose [Leonard 2001]. If plasma ammonia is not stabilized by dietary restriction alone, ammonia scavengers that promote an alternative pathway of nitrogen removal can be administered [Batshaw 2001; Häberle 2012; Lichter-Konecki 2013; Häberle 2019]. Ammonia scavengers cannot, however, completely prevent individuals from having hyperammonemic crises [Batshaw 2001]. Orthotopic liver transplantation can correct OTC deficiency; however, this is limited by donor availability and is associated with significant risk of morbidity and mortality [Leonard 2004].

Thus, there remains a significant unmet medical need for a treatment that allows for sustained ammonia management and prevention of hyperammonemic crises associated with OTC deficiency.

1.1 Adeno-Associated Viral Vectors

Adeno-associated virus (AAV) is a nonenveloped, icosahedral, single-stranded DNA virus. Given that wild-type AAV displays wide tissue tropism and is capable of persisting in tissues for long durations without pathogenic effects, the use of recombinant AAV vectors has become a popular tool for gene delivery. Additionally, recombinant AAV vectors are nonreplicating and the vector genomes exist as an episome following tissue transduction, minimizing the risk of insertional mutagenesis [Nakai 2001]. Since the first genetic engineering of wild-type AAV as a gene delivery vector in the early 1980s, recombinant AAV has shown great promise as an effective and safe gene delivery vehicle for treatment of diseases [Gao 2005].

Adeno-associated virus serotype 2 (AAV2) was the first AAV that was used for gene transfer applications and has been used in numerous studies for a variety of diseases such as alpha 1-antitrypsin deficiency, Batten disease, and cystic fibrosis [Mingozzi 2011]. In animal models, AAV-mediated gene transfer was shown to treat the underlying disease for many years [Snyder 1999; Mount 2002; Wang 2005; Nichols 2010]. However, in humans, AAV2-mediated delivery was either sub-therapeutic or only lasted a few months [Manno 2003; Manno 2006]. This was attributed to several limitations of AAV2 vectors including low transduction efficiency [Yan 2002], high seroprevalence of neutralizing antibodies against AAV2 in humans [Boutin 2010], and potentially destructive T-cell responses to capsids [Gao 2009; Vandenberghe 2006; Wang 2007]. Additionally, there has been evidence of B-cell responses against the transgene product in an animal model of hemophilia [Herzog 2001].

1.2 Selection of the AAV Clinical Candidate

The host immune response (ie, neutralizing antibodies and T-cell responses) limiting the efficacy of AAV2-mediated gene transfer (Section 1.1) was not predicted in the initial animal studies, due to AAV2 not being endemic in the nonhuman animal species under investigation. Novel AAV serotypes, isolated from nonhuman primates, are divergent enough from endemic human serotypes to circumvent the neutralizing antibodies existing in most human subjects while retaining similar tissue tropism [Gao 2002].

One of these serotypes, AAV serotype 8 (AAV8), displays strong tropism for the liver [Gao 2002] and has been tested extensively as a vector for gene transfer in nonclinical and clinical models of hemophilia [Davidoff 2005; Jiang 2006; Nathwani 2006; Nathwani 2007; Nathwani 2011a].

These studies have shown that AAV8 has clear advantages over AAV2 that include the following: excellent transduction efficiency; liver-specific tropism; stable transgene expression; a lack of hepatotoxicity as measured by peak serum aminotransferases; a lack of liver histopathology findings; a lack of T-cell activation to the transgene product; and low levels of pre-existing neutralizing antibodies in human populations, minimizing their inhibition of in vivo transduction. Furthermore, in clinical studies, AAV8 has shown impressive efficacy and safety for the treatment of hemophilia B [Nathwani 2011a; Nathwani 2014] and is currently being studied for the treatment of glycogen storage disease type Ia (NCT03517085), hemophilia A (NCT03001830), hemophilia B (NCT00979238 and NCT01687608), X-linked retinoschisis (NCT02317887), human immunodeficiency virus-1 (NCT03374202), X-linked retinitis pigmentosa (NCT03116113), X-linked myotubular myopathy (NCT03199469), homozygous familial hypercholesterolemia (NCT02651675), Pompe disease (NCT03533673), mucopolysaccharidosis disease (NCT03173521), and achromatopsia (NCT03758404 and NCT03001310).

The study product, DTX301, contains a codon-optimized human wild-type OTC gene with expression driven by both a liver-specific enhancer element and a liver-specific promoter encapsidated within a nonreplicating AAV8 vector. In *spf*^{ash} mice, which is an established nonclinical model of OTC deficiency that results from a missense mutation in exon 4 of the OTC gene [Hodges 1989], this vector showed a sustained, dose-dependent improvement in both OTC expression and activity following a single intravenous (IV) injection that was comparable to wild-type mice [Wang 2012]. These data provide support for the feasibility of this therapeutic approach in humans.

This clinical study differs from previous gene transfer studies for OTC in a number of ways:

1. Adeno-associated virus is used as the vehicle for gene delivery. Unlike adenovirus, which had limited efficacy and significant toxicity at high doses in previous OTC gene transfer studies [Raper 2002; Raper 2003], AAV vectors have shown impressive safety and long-term efficacy in a number of diseases [Kay 2011; Nathwani 2011a; Nathwani 2014];
2. The liver is the natural site of ureagenesis. The AAV8 serotype demonstrates high liver tropism and can achieve efficient liver gene transfer following IV infusion [Gao 2002];
3. The AAV8 serotype is not thought to be endemic in the human population and, therefore, the prevalence of pre-existing neutralizing antibodies to AAV8 in patients with OTC deficiency is expected to be lower than other AAV serotypes, such as AAV2 [Calcedo 2013].

1.3 Study Rationale

Ornithine transcarbamylase gene transfer is expected to be effective for the treatment of OTC deficiency since the disease is caused by mutations within a single gene [Caldovic 2015]. Unlike current medical treatment options (dietary restriction and ammonia scavengers), OTC gene transfer offers the potential to correct the underlying deficiency for a prolonged period with a single IV infusion. Currently, no gene transfer product has been approved for the treatment of OTC deficiency. Increasing OTC activity and promoting the removal of ammonia through the urea cycle should allow patients with OTC deficiency to avoid hyperammonemic crises in the context of a well-controlled diet, while reducing or stopping ammonia scavenger therapy. Furthermore, depending on the level of OTC expression and activity achieved, patients may be able to loosen their dietary restrictions, which should greatly improve their quality of life (QoL).

1.3.1 Design Rationale

The design of this study is consistent with global regulatory guidelines for protocol design, including: subject selection, dose estimation, precautions applied between dosing cohorts, risk mitigation, and study stopping criteria.

Study 301OTC01 is a Phase 1/2, open-label, multicenter, safety and dose-finding study to determine the safety, tolerability, and preliminary efficacy of DTX301 in adults with late-onset OTC deficiency. The primary objective of the study is to determine the safety and tolerability of single doses of DTX301. A key element of the study is the identification of the optimal biological dose (OBD) of DTX301. The secondary objectives of the study are the assessment of rate of ureagenesis, reflecting the direct *in vivo* efficiency of the urea cycle and the assessment of plasma ammonia ($AUC_{0\text{-}24}$), reflecting clinical metabolic control. The target for DTX301 is to achieve a rate for ureagenesis of

approximately 300 µmol/kg/hr (±10%), which is the approximate rate of ureagenesis in healthy adults [Matthews 1984; Jahoor 1987; Castillo 1996; Tuchman 2008b].

With regards to defining clinically meaningful hyperammonemia as ≥ 100 µmol/L in this study (Section 4), a plasma ammonia level of ≥ 100 µmol/L is widely accepted in both clinical practice and published literature as a clinically significant level where OTC patients are likely to be symptomatic (including neuropsychological impairments) due to hyperammonemia [Lichter-Konecki 2013]. Furthermore, plasma ammonia exceeding 100 µmol/L is identified by the United Kingdom National Metabolic Biochemistry Network as clinically elevated [Wright 2010] and is associated with an increased risk of death (odds ratio 1.5 or greater) in OTC patients [Ozanne 2012]. Published guidelines for the management of hyperammonemia in UCDs also recommend restarting enteral feeding once plasma ammonia is < 100 µmol/L [Häberle 2012; Häberle 2019].

Consistent with the UCDC, we have defined symptomatic hyperammonemic events, classified as hyperammonemic crisis (HAC) throughout the study, as an episode of signs and symptoms associated with hyperammonemia (such as frequent vomiting, nausea, headache, lethargy, irritability, combativeness, and somnolence) with documented elevated ammonia levels (≥ 100 µmol/L) and requiring medical intervention [Kent and Holt 2017; Longo and Holt 2017; Diaz 2019].

Eligible subjects will receive a single IV infusion of DTX301. Three subjects will be enrolled in Cohort 1 and a minimum of 2 to 3 subjects will be enrolled in each subsequent cohort; the number of subjects in each subsequent dosing cohort will be determined by subject results and availability of study product. Subjects in Cohorts 1 and 2 will be dosed at a minimum of 2 weeks (14 days) apart. The initial 3 subjects in Cohort 3 will be dosed at a minimum of 1 week apart (7 days). Additional subjects to Cohort 3 (Cohort Expansion) and subjects to Cohort 4 (Dosing Process Optimization) may be dosed less than 1 week apart. There will be a minimum of 12 weeks (84 days) followed by DMC review of a dosing cohort between the dosing of the last subject in one dosing cohort and the first subject in the next dosing cohort up to Cohort 3. Dosing of additional subjects as expansion of Cohort 3, and initiation of Cohort 4 (Dosing Process Optimization) may occur in parallel, following the DMC review of a minimum of 12 weeks of data from the initial 3 subjects in Cohort 3. Subjects may be assigned to either expansion of Cohort 3 or Cohort 4, with at least 1 male subject assigned to each. A continual reassessment method (CRM) will be used for dose finding to discover the OBD of DTX301 (Section 10.5.1). Cohort 4 is intended to dose at the OBD.

The proposed dosing interval of 14 days between subjects in the first and second dose cohorts, and 7 days between each of the first 3 subjects in Cohort 3, followed by a shorter dosing interval in the expansion of Cohort 3 and Cohort 4, is supported by the safety profile and lack of serious adverse

reactions reported in AAV-mediated gene transfer in human subjects, including those using AAV8 vector in a hemophilia B clinical study [Manno 2006; Nathwani 2011a; Nathwani 2014], and the ongoing safety review from this study, which includes DMC assessment [Diaz 2019; Ultragenyx Pharmaceutical, data on file]. Moreover, an ongoing Phase 1/2 clinical study in hemophilia B using AAV-mediated gene transfer at a starting dose of 5.0×10^{12} genome copies (GC)/kg (higher than the proposed DTX301 starting dose of 3.0×10^{12} GC/kg) at multiple European study sites is dosing subjects within a cohort at a minimum of only 1 day (24 hours) apart [uniQure 2014; Clinicaltrials.gov identifier: NCT02396342; EudraCT Number: 2013-005579-42].

The most common product-related AE observed in clinical studies with AAV-mediated gene transfer to subjects with moderate to severe hemophilia B has been an asymptomatic transient rise in liver aminotransferases (still within the normal reference range for these laboratory parameters) and concurrent decline in transgene expression approximately 7 to 10 weeks following vector administration [Manno 2006; Nathwani 2011a; Nathwani 2014]. In all cases, the transient rise in liver aminotransferases resolved without clinical sequelae. It has been hypothesized that this vector-induced hepatitis is due to the activation of capsid-specific cytotoxic T lymphocytes and destruction of transduced liver cells [Mingozzi 2007]. As this is the only identified AE with AAV gene transfer and, given that subjects in this study will be closely monitored for vector-induced hepatitis and treated with corticosteroids (Section 8.2.4.1.2), having an interval of more than 14 days between subjects within Cohorts 1 and 2 and 7 days between the first 3 subjects in Cohort 3 does not change either safety evaluations or the medical management of study subjects. To date, no infusion-related or serious AEs (SAEs) have been reported with DTX301. Aligned with previous experience [Manno 2006; Nathwani 2011a; Nathwani 2014], only mild, transient, asymptomatic rises in liver enzymes have been observed in 6 subjects starting approximately 8 days to 7 weeks after vector administration that resolved with an oral corticosteroid taper regimen [Diaz 2019; Ultragenyx Pharmaceutical, data on file;]. To allow for refinement in management of asymptomatic transient rises in liver aminotransferases (vector-induced hepatitis) and concurrent decline in transgene expression following AAV vector administration, Cohort 4 (Dosing Process Optimization) will utilize an alternative regimen of oral corticosteroids. Based on available evidence, it is expected that vector-induced hepatitis will be self-limiting. Therefore, oral prednisone (or prednisolone) will be employed in a prophylactic regimen initiated before dosing with DTX301, sustained for 4 weeks, followed by tapering (Section 8.2.4.1.2). Prophylactic corticosteroid regimens are in use for gene transfer therapies [Audentes 2017 Clinicaltrials.gov identifier: NCT03223194; Abeona Therapeutics 2016 ClinicalTrials.gov identifier: NCT02716246].

Subject safety will be closely monitored throughout the study, including assessment of hyperammonemic crises, assessment of metabolic and clinical stability, frequent monitoring of liver

enzymes and plasma ammonia levels, and inpatient observation following dosing and at multiple time points throughout the 52-week study duration.

After all subjects in Cohort 1 and Cohort 2, and the initial 3 subjects in Cohort 3 have completed Week 12, a data monitoring committee (DMC) will meet to review safety data (ie, AEs/SAEs, physical examination findings, vital sign measurements, electrocardiogram [ECG] results, and longitudinal clinical laboratory assessments [including assessment of liver function and plasma ammonia]) and provide a recommendation for progressing to the next dosing level, as applicable (Section 9.3), and enrollment of additional subjects to Cohort 3 (Cohort Expansion) and subjects to Cohort 4 (Dosing Process Optimization). If an investigator reports an AE/SAE that meets any of the study stopping criteria (Section 3.2.7), the DMC will meet on an ad hoc basis to review and assess the event. The DMC may, at any time, recommend modifying or pausing enrollment due to safety concerns based on these periodic data reviews. Additional DMC meetings may take place, as needed. The full scope of each review is outlined in the DMC charter.

Subjects will be followed for 52 weeks after dosing. After completion of this study, subjects will be asked to enroll in a 4-year extension study to evaluate the long-term (a total of 5 years after dosing) safety and efficacy of DTX301.

1.3.2 Dosing Rationale

The current working estimation to determine the clinical starting dose detailed below is based on available nonclinical data with DTX301, including the minimal effective dose (MED) and optimal effective dose of DTX301 in mice, clinical experience with other AAV8 products, and known limitations when scaling between species.

Based on preliminary nonclinical study results of DTX301 [redacted]

In order to estimate the allometric scaling needed for AAV8-mediated delivery of the OTC transgene in this study, two data sets using AAV-mediated transfer of human factor IX were examined: the uniQure (Amsterdam, The Netherlands) data set [uniQure 2015] and the Nathwani data set [Nathwani 2007; Nathwani 2011a; Nathwani 2011b]. The AAV5 vector used in the uniQure studies was shown to have a 10-fold scaling between mouse and human [uniQure 2015]. The AAV8 vector used in the

Nathwani studies supports a 10-fold scaling from mouse to nonhuman primates and a 4-fold scaling between nonhuman primates and humans, for a total of 40-fold between mouse and human [Nathwani 2007; Nathwani 2011a; Nathwani 2011b]. Therefore, when considering a clinical dosing rationale, in light of the available comparison data between mice and humans, it seems reasonable to consider a 10-fold to 40-fold increase in dose from mice to humans to achieve similar target levels for the expression and activity of OTC. [redacted]

In comparison to the clinical development of traditional small molecule and biologic therapies, clinical studies for AAV-mediated gene transfer have an additional consideration; after initial dosing, it is expected that subjects who are administered DTX301 will develop neutralizing antibodies to AAV8 and will receive no benefit from subsequent dosing with this vector. Therefore, unlike classic dose-escalation studies, there is a greater focus on targeting efficacy in addition to safety. [redacted] consistent with US Food and Drug Administration (FDA) Guidance for Industry on the Considerations for the Design of Early Phase Clinical Trials of Cellular and Gene Therapy Products [CBER 2015].

Another consideration is that the maximal feasible dose of vector that can be administered to mice in Good Laboratory Practice toxicology studies (based on dose volume and concentration of vector) is 7.0×10^{13} GC/kg. [redacted]

The starting dose for this study (2.0×10^{12} GC/kg) [redacted] which is anticipated to be the no observed adverse effect level. [redacted] below the highest dose used in published nonhuman primate studies with AAV8 (2×10^{13} GC/kg) [Jiang 2006; McIntosh 2013] and is similar to the doses for other AAV-mediated gene transfer

products administered to humans of 2×10^{12} vector genomes/kg (AAV8) [Nathwani 2011a; Nathwani 2014] and 5×10^{12} GC/kg (AAV5) [uniQure 2014]. [redacted] GC/kg will provide a reasonable probability that all subjects in this study have the potential for a clinically substantial benefit from DTX301.

An adaptive design for dose finding is being used in this study. The CRM will model the probability of experiencing a dose-limiting toxicity (DLT) in subsequent dosing cohorts using cumulative safety data (Section 10.5.1 and Figure 10-1). [redacted] Additionally, depending on the results, additional subjects at the dose of interest may be recruited to confirm the OBD.

Based on existing safety data from clinical studies using AAV-mediated gene transfer (including multiple hemophilia programs), substantial toxicity in the predicted therapeutic range is not anticipated. Ultragenyx Pharmaceutical is also taking into account the amount of DTX301 Chemistry, Manufacturing, and Controls material that can be produced over the anticipated timeline of this Phase 1/2 study. Assuming that a maximum tolerated dose (MTD) is not identified in nonclinical studies, the proposed dose-finding approach for this Phase 1/2 study is consistent with the FDA Guidance for Industry on the Considerations for the Design of Early Phase Clinical Trials of Cellular and Gene Therapy Products that achieving a specified target range of exposure is the focus of a cellular or gene therapy program when "there are significant practical limits of the dose of the product that can be produced or delivered" [CBER 2015].

For additional nonclinical information, please refer to the DTX301 investigator's brochure.

2 Study Objectives and Endpoints

Objective	Endpoint
Primary	
To determine the safety of single IV doses of DTX301 in adults with late-onset OTC deficiency.	The incidence of AEs, TEAEs, and SAEs for each dosing cohort, assessed by severity and relationship to study product.
Secondary	
To establish a dose of DTX301 that has a meaningful increase in the rate of ureagenesis to allow further clinical development.	The change from baseline in the rate of ureagenesis (as measured by the generation of [^{13}C]urea over 4 hours) as determined by gas chromatography mass spectrometry over time to 52 weeks after the IV administration of DTX301.
To evaluate the efficacy of single IV doses of DTX301 in adults with late-onset OTC deficiency in the setting of tapering or discontinuing ammonia scavenger medications.	The change from baseline (Day 0) in plasma ammonia area under the curve from time zero to 24 hours (AUC_{0-24}) over time to 52 weeks after IV administration of DTX301 in the setting of tapering or discontinuing ammonia scavenger medications.
Exploratory	
To assess the impact of DTX301, by dose, on the number of hyperammonemic crises during the study.	The number of hyperammonemic crises observed for each dose over time to 52 weeks after IV administration of DTX301.
To evaluate the effect of DTX301, by dose, on urinary orotic acid levels.	The change from baseline in urinary orotic acid excretion over time to 52 weeks after IV administration of DTX301.
To evaluate the effect of DTX301, by dose, on plasma glutamine and glutamate levels.	The change from baseline in serum glutamine and glutamate over time to 52 weeks after IV administration of DTX301 .
To assess the impact of DTX301, by dose, on the subject's neuropsychological functioning.	Changes in responses to the following, summarized by dose level of DTX301: [redacted]
To assess the impact of DTX301, by dose, on the subject's quality of life.	Responses to the PROMIS questionnaire, summarized by dose level of DTX301 over time to Week 52.
To assess the impact of DTX301, by dose, on the use of ammonia scavengers.	Use of ammonia scavengers, summarized by dose level of DTX301.
To assess the impact of DTX301, by dose, on dietary protein restriction.	Change in dietary protein intake, summarized by dose level of DTX301.
To describe the immune response to AAV8 capsid	• The development of neutralizing antibodies to

Objective	Endpoint
proteins after IV administration of DTX301.	AAV8 (as determined by a cell-based assay), summarized by time point and dose level of DTX301. • The development of anti-AAV8 binding antibodies (as determined by ELISA), summarized by time point and dose level of DTX301.
To describe the immune response to OTC after IV administration of DTX301.	• The development of anti-OTC antibodies (as determined by ELISA) summarized by time point and dose level of DTX301.

Abbreviations: AAV8, adeno-associated virus serotype 8; AE, adverse event; AUC_{0-24}, area under the curve from time zero to 24 hours; ELISA, enzyme-linked immunosorbent assay; IV, intravenous; OTC, ornithine transcarbamylase; PROMIS, Patient-Reported Outcomes Measurement Information System; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

3 Investigational Plan

3.1 Study Overview

Study 301OTC01 is a Phase 1/2, open-label, multicenter, safety and dose-finding study to determine the safety, tolerability, and preliminary efficacy of DTX301 in adults with late-onset OTC deficiency. A key element of the study is the identification of the OBD for DTX301.The primary objective of the study is to determine the safety of single doses of DTX301. The secondary objectives of the study are the assessment of rate of ureagenesis, reflecting the direct *in vivo* efficiency of the urea cycle and the assessment of plasma ammonia ($AUC_{0\text{-}24}$), reflecting clinical metabolic control. The target for DTX301 is to achieve a rate for ureagenesis of approximately 300 µmol/kg/hr (±10%), which is the approximate rate of ureagenesis in healthy adults [Matthews 1984; Jahoor 1987; Castillo 1996; Tuchman 2008b].

Eligible subjects will receive a single IV infusion of DTX301. Three subjects will be enrolled in Cohort 1 and a minimum of 2 to 3 subjects will be enrolled in each subsequent cohort. Dose escalation will be conducted according to a model that uses safety data to predict the OBD (Section 10.5.1, Figure 10-1, and Figure 10–2).

The following candidate doses (per kilogram of body weight) will be used to determine the OBD (Section 10.5.1):

- 2.0×10^{12} GC/kg
- 6.0×10^{12} GC/kg
- 1.0×10^{13} GC/kg

There will be a minimum of 14 days between dosing of each subject in Cohorts 1 and 2 and 7 days between dosing of the initial 3 subjects in Cohort 3. Additional subjects to Cohort 3 (Cohort Expansion) and subjects to Cohort 4 (Dosing Process Optimization) may be dosed less than 1 week apart.

Dose escalation will be conducted according to a model that uses the collected data to predict the safety profile of the dose in order to determine the OBD. There will be a minimum of 12 weeks followed by data monitoring committee (DMC) review (Section 9.3) of a dosing cohort between the dosing of the last subject in one dosing cohort and the first subject in the next dosing cohort up to Cohort 3. Dosing of additional subjects as expansion of Cohort 3, and initiation of Cohort 4 (Dosing Process Optimization) may occur in parallel, following the DMC review of a minimum of 12 weeks

of data from the initial 3 subjects in Cohort 3. A CRM will be used for dose finding to discover the OBD of DTX301. Cohort 4 is intended to dose at OBD. There will be no intra-cohort dose escalations.

Subjects treated in Cohort 4 (Dosing Process Optimization) will utilize an alternative regimen of corticosteroids, aiming at prophylaxis of vector-induced hepatitis. Therefore, oral prednisone (or prednisolone) will be initiated before dosing with DTX301, sustained for 4 weeks, followed by tapering (Section 8.2.4.1.2).

Enrollment will be stopped, and the regulators will be notified if, at any time during the study, any of the safety stopping criteria are met (Section 3.2.7).

Subjects will be followed for 52 weeks after dosing. After completion of this study, subjects will be asked to enroll in a 4-year extension study to evaluate the long-term (a total of 5 years after dosing) safety and efficacy of DTX301.

3.2 Overall Study Duration and Follow-Up

The duration of the study is defined for each subject as the date that written informed consent is provided through the visit at Week 52. Subjects will be in the study for approximately 56 weeks (including the screening period).

The study is anticipated to enroll up to 18 subjects at approximately 20 study sites globally.

All study visits, clinic or home visits, and the timing of assessments are detailed in Table 15-1 and Table 15-2. Subjects will be asked to participate in 20 outpatient clinic or home visits (approximately 1.5 to 5 hours in length depending on the assessments performed at the visit) and 5 inpatient clinic visits (approximately 24 to 48 hours in length). Where available, agreed upon by the investigator and allowed by local regulation, an outpatient clinic visit may take place as home health services. **Investigators will be responsible for maintaining effective lines of communication between the investigator and the personnel who manage the subjects at home to guarantee that the investigator is constantly kept informed of subject safety, including metabolic and clinical status.**

A schematic of the study design is provided in Figure 3-1.

Figure 3-1 Study Design

Screening Period (Day –35 to Day –1)		
Determine subject eligibility Clinical and specialty labs and ECG Determine rate of ureagenesis (administer [1-^{13}C]sodium acetate) Review ammonia scavenger use and dietary protein restrictions Subjects participating in Cohort 4 (Dosing Process Optimization) will start prophylactic corticosteroid regimen at least 5 days prior to dosing with DTX301		
Baseline (Day 0 through Day 1) – Inpatient		
Approximately 48-hour inpatient stay • Clinical and specialty labs and ECG • PROMIS questionnaire and neuropsychological tests, including a brief IQ test (WASI-II vocabulary and matrix reasoning subtests) • AUC_{0-24} plasma ammonia and 24-hour urinary orotic acid determination (Day 0) • Determine rate of ureagenesis (administer [1-^{13}C]sodium acetate on Day 1) • Administer DTX301 (on Day 1 following completion of ureagenesis measurement) • Discharge (24 hours after DTX301 administration) Subjects participating in Cohort 4 (Dosing Process Optimization) will start prophylactic corticosteroid regimen at least 5 days prior to dosing with DTX301.		
Treatment Period		
Dose Cohort	**Outpatient Clinic Visits (Weeks 2, 4, 10, 16, 20, and 36)**	**Inpatient Visits (approximately 28-hour) (Weeks 6, 12, and 24)**
1 to 3	Clinical and specialty labs	Clinical and specialty labs
	Viral shedding (saliva, urine, and stool)	Viral shedding (saliva, urine, and stool)
	Vector genome sample (blood)	Vector genome sample (blood)
	Orotic acid spot urine	PROMIS questionnaire
	Rate of ureagenesis (administer [1-^{13}C]sodium acetate at Week 20 only).	Plasma ammonia AUC_{0-24}
		Urinary orotic acid (every 6 hours over 24 hours)
		Determine rate of ureagenesis (administer [1-^{13}C]sodium acetate)
4 and expansion of 3	**Outpatient Clinic or Home Health Service Visits (Weeks 2, 4, 10, 16, and 36)**	**Inpatient Visits (approximately 28-hour) (Weeks 6, 12, and 24)**
	Clinical labs	Clinical and specialty labs
	Orotic acid spot urine	Viral shedding (saliva, urine, and stool)
	Outpatient Clinic or Home Health Service Visits (Weeks 10, 16, and 36)	PROMIS questionnaire
	Viral shedding (saliva, urine, and stool)	Plasma ammonia AUC_{0-24}
	Outpatient Clinic (Weeks 20)	Urinary orotic acid (every 6 hours over 24 hours)
	Rate of ureagenesis (administer [1-^{13}C]sodium acetate); clinical labs, orotic acid spot urine, and viral shedding (saliva, urine, and stool)	.
• There will be a minimum of 14 days between subjects in Cohorts 1 and 2, and a minimum of 7 days between subjects between dosing of the initial 3 subjects in Cohort 3. Additional subjects to Cohort 3 (Cohort Expansion) and subjects to Cohort 4 (Dosing Process Optimization) may be dosed less than 1 week apart. • There will be a minimum of 12 weeks followed by data monitoring committee (DMC) review of a dosing cohort between the dosing of the last subject in one dosing cohort and the first subject in the next dosing cohort up to Cohort 3. • Dosing of additional subjects as expansion of Cohort 3, and initiation of Cohort 4 (Dosing Process Optimization) may occur in parallel, following the DMC review of a minimum of 12 weeks of data from the initial 3 subjects in Cohort 3.		

• Subjects treated in Cohort 4 (Dosing Process Optimization) will utilize an alternative regimen of corticosteroids, aiming at prophylaxis of vector-induced hepatitis. Therefore, oral prednisone (or prednisolone) will be initiated at least 5 days before dosing with DTX301, sustained for 4 weeks, followed by tapering. • Where available, agreed upon by the investigator, and allowed by local regulation, an outpatient clinic visit may take place as home health services.
End of Study (Week 52)/Early Withdrawal – Inpatient
Approximately 28-hour inpatient stay • Clinical and specialty labs and ECG • PROMIS questionnaire and neuropsychological tests • AUC_{0-24} plasma ammonia and 24-hour urinary orotic acid determination • Determine rate of ureagenesis (administer [1-^{13}C]sodium acetate) • Discharge following completion of ureagenesis measurement • Subject will be asked to enroll in a long-term (4-year) extension study • Review ammonia scavenger use and dietary protein restrictions

Abbreviations: AUC_{0-24}, area under the curve from time zero to 24 hours; DMC, data monitoring committee; IQ, intelligence quotient; PROMIS, Patient-Reported Outcomes Measurement Information System; WASI-II, Wechsler Abbreviated Scale of Intelligence, Second Edition.

3.2.1 Screening Period

After a subject has provided written informed consent, the investigator or other qualified study personnel will determine if the subject is eligible for the study. This will be accomplished by reviewing the inclusion and exclusion criteria (Section 4) and completing all of the screening assessments outlined with corresponding timeframe guidance in the Schedule of Events (Table 15-1). Screening should be concluded within 35 days before Day 0. NOTE: The screening assessments may be performed on more than 1 day; all assessments must be completed and results available and reviewed prior to Day 0.

At Screening, blood and urine will be collected. Subjects will be administered [1-^{13}C]sodium acetate orally for the determination of ureagenesis as outlined in Section 8.3.1. The assessment of rate of ureagenesis may be repeated during Screening if discrepant with subject's clinical status and severity.

The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable in order to receive [1-^{13}C]sodium acetate. If the ammonia level is inconsistent with a subject's clinical status, the ammonia level may be repeated to ensure accurate results. If the subject is deemed clinically unstable, administration of [1-^{13}C]sodium acetate dosing will be held. Blood samples for determination of ureagenesis will be collected, via an indwelling catheter, before dosing (time 0) and at approximately 0.5, 1, 1.5, 2, 3, and 4 hours after dosing with [1-^{13}C]sodium acetate.

Subjects who screen fail due to one or more criteria that may potentially be resolved or reversed over time in the opinion of the investigator (for example, certain laboratory values or presence of active infection), or whose OTC deficiency is temporarily metabolically or clinically unstable, may be

rescreened one time. Subjects may be rescreened after being clinically and metabolically stable for at least 28 days.

If rescreening is within 3 months of the original screening, the following must be repeated: hematology, coagulation and urinalysis, serum pregnancy test (if applicable), clinical chemistry, plasma ammonia, amino acid panel, AAV8 neutralizing antibody test, weight, and vital signs. Additional tests may be requested on a case-by-case basis, depending on the original reason for screen failure. The AAV8 screening neutralizing antibody results must be available and reviewed before IP administration. If the rescreening occurs more than 3 months after the original screening, all tests must be repeated except the following: OTC genotyping, HBV, HCV, and HIV.

3.2.2 Baseline Period

Subjects will be admitted to the inpatient clinic on Day 0 and will be required to stay for approximately 48 hours. While the timing of assessments during the Baseline Visit outlined below reflects the most time efficient approach, individual centers may conduct alternate or extended timing and sequence of assessments that meet requirements, prior to dosing with DTX301.

3.2.2.1 Baseline Visit – Day 0

The timing of all assessments and procedures for Day 0 is outlined in the Schedule of Events (Table 15-1). On Day 0, the baseline determination of plasma ammonia for area under the curve from time zero to 24 hours (AUC_{0-24}) and urinary orotic acid levels (over 24 hours) will be performed. Samples for the determination of plasma ammonia (AUC_{0-24}) and urinary orotic acid levels over 24 hours will be collected prior to the oral administration of [1-^{13}C]sodium acetate on Day 1. Blood and urine will be collected as outlined in Section 8.3.

3.2.2.2 Baseline – Day 1

The timing of all assessments and procedures for Day 1 is outlined in the Schedule of Events (Table 15-1). The collection of samples for the determination of plasma ammonia (AUC_{0-24}) and urinary orotic acid levels over 24 hours need to be completed prior to the determination of ureagenesis. Subjects will be administered [1-^{13}C]sodium acetate orally for the determination of ureagenesis. Blood and urine will be collected as outlined in Section 8.3.

Following completion of sampling for ureagenesis (ie, the 4-hour time point), subjects will then receive a single, peripheral IV infusion of DTX301. Safety (eg, vital sign measurements [Section 8.2.2], ECGs [Section 8.2.3], clinical laboratory assessments [Section 8.2.4], and AEs

[Section 9.1]) will be monitored for 24 hours following infusion of DTX301 (Table 15-1). Subjects will be discharged after a 24-hour observation period. Any AEs that occur during this 24-hour observation period will be treated with appropriate supportive and medical care deemed necessary for the well-being of the subject.

Prior to the start of DTX301 infusion, the study site must confirm that the subject's plasma ammonia level on Day 1 (predose) is < 100 µmol/L for patients who historically maintain normal ammonia levels, and the subject is clinically stable; OR subject's plasma ammonia level on Day 1 (predose) is < 200 µmol/L, for patients who historically are not able to fully control ammonia levels with baseline management, and the subject is clinically stable. If the Day 1 (predose) ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. The 24-hour time point (T 24) from local laboratory (STAT sample) for (AUC_{0-24}) of plasma ammonia can serve as the DTX301 predose plasma ammonia result, if drawn within 12 hours or less of dosing with DTX301. If not, a new plasma ammonia (STAT sample) is to be collected prior to dosing with DTX301. **The predose plasma ammonia used to determine DTX301 dosing must be drawn within 12 hours or less of dosing.** If the subject is deemed clinically unstable, dosing will be held, and the subject can be rescreened once the subject is determined to be clinically stable. Repeat of screening assessments or rescreen process may take place depending on timing and subject clinical status (Section 3.2.1 and Table 15-1).

3.2.3 Treatment Period

Subjects will be evaluated approximately every 4 days through Week 12 at home, at outpatient clinic visits, or as inpatient visits (Table 15-1 and Table 15-2). Following the Week 12 visit, subjects will visit the study site once approximately every 4 weeks through Week 24, at Week 36, and at Week 52 or their early withdrawal from the study (Table 15-1 and Table 15-2). Where available, agreed upon by the investigator and allowed by local regulation, an outpatient clinic visit may take place as home health services. **Week 20 visit remains a mandatory outpatient clinic visit for all subjects.**

At any point between scheduled visits, additional unscheduled assessment of LFTs, plasma ammonia, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator.

3.2.3.1 Home or Clinic Visits

Subjects will be asked to provide clinical laboratory samples approximately every 4 days through Week 12 of the study. Through Week 12, one sample for spot ammonia will be collected

approximately once a week, and sent to the local laboratory (STAT sample). If subjects cannot visit the study site in person, they may have the option of being visited by clinically trained and qualified personnel (if available) approximately every 4 days at their home through Week 12 (Table 15-2), the exception being required study site visits when the subject must visit the study site (Table 15-1). Saliva, urine, and stool samples will also be collected at these visits (through Week 12) for assessment of viral shedding (Table 15-2) and will continue to be collected until 3 consecutive negative results are obtained from each matrix (Section 8.2.5.3).

3.2.3.2 Outpatient Clinic or Home Health Service Visits

Subjects will visit the study site on an outpatient basis at Weeks 2, 4, 10, 16, 20, and 36 for efficacy and safety assessments as well as the determination of ureagenesis (at Week 20 only) as outlined in the Schedule of Events (Table 15-1). Where available, agreed upon by the investigator and allowed by local regulation, an outpatient clinic visit may take place as home health Services. **Week 20 visit remains a mandatory outpatient clinic visit for all subjects.**

3.2.3.3 Inpatient Visits

Subjects will be admitted to the study site at Weeks 6, 12, 24, and 52 for the determination of plasma ammonia (AUC_{0-24}), urinary orotic acid levels, and ureagenesis. Samples for the determination of plasma ammonia (AUC_{0-24}) and urinary orotic acid levels will be collected over the 24-hour period prior to the oral administration of [1-^{13}C]sodium acetate. Blood and urine will be collected as outlined in Section 8.3.

The timing of all assessments and procedures for each visit is outlined in the Schedule of Events (Table 15-1). Subjects will be discharged following the 4-hour time point for ureagenesis, after all assessments/samples have been completed/collected. Subjects will be housed for approximately 28 hours.

3.2.4 Tapering of Ammonia Scavenger Therapy

There are multiple types of ammonia scavenger medications available globally and patients with OTC deficiency typically have a personalized prescription of one more ammonia scavenger medications to optimize the management of their disease.

Adjustments to ammonia scavenger therapy may be considered following the Week 12 and Week 24 visits. Changes to baseline treatment (ie, ammonia scavenger therapy and protein-restricted diet)

cannot occur until there is evidence of transgene expression reflected by continued evidence of metabolic stability with ammonia levels in the normal range or improved ammonia control if subjects have a history of ammonia levels above the upper limit of normal and supported by improvement in clinical signs and symptoms. The subject must be clinically stable and under good metabolic control before changes can be initiated or progressed. The risks of making adjustments to baseline treatment on their own, without express guidance from the site, will be reinforced with the subject at site visits.

Changes to baseline treatment cannot occur while the subject is treated with corticosteroids or within a 2-week period of completing a corticosteroid taper.

Modification of ammonia scavenger therapy cannot occur at the same time as changes in protein-restricted diet. Once medications or diet are adjusted or discontinued, the subject must demonstrate good metabolic control prior to adjustments in the other type of baseline treatment.

Modification of baseline treatment will be individualized, dependent on the clinical judgment of the investigator, and based on review of the totality of longitudinal clinical and laboratory data for each subject, including spot plasma ammonia levels, plasma ammonia (AUC_{0-24}), subject clinical stability/asymptomatic status, neurocognitive status, and subject-reported outcomes. **Rate of ureagenesis cannot be used for decision-making in modification of ammonia scavenger therapy or protein-restricted diet and results will not be made available to the investigative sites until the end of the study.** During periods of adjustment to baseline treatment subjects will be closely monitored.

At any time after modification and/or discontinuation of ammonia scavenger therapy, reinstitution of therapy may take place based on the subject's clinical and metabolic status (elevated ammonia levels or signs and symptoms consistent with hyperammonemia), and under evaluation of the investigator.

Reinstitution of ammonia scavenger therapy should be considered if:

1. Subjects demonstrate 2 or more documented consecutive laboratory results of hyperammonemia (plasma ammonia level ≥ 100 µmol/L). NOTE: If the subject is clinically stable, spot ammonia may be repeated if discrepant with subject's clinical status and severity, OR

2. Subjects demonstrate upward trending plasma ammonia levels less than 100 µmol/L, associated with clinical signs or symptoms suggestive of worsening underlying disease, OR

3. Subjects experience a time of increased metabolic demand, such as intercurrent viral infection, treatment with steroids, hyperammonemia, or hyperammonemic crisis (Section 3.2.6).

After reinstitution of ammonia scavenger therapy, once intercurrent illness is resolved (if applicable), and the subject is documented as clinically and metabolically stable, a second round of modification of baseline treatment can be considered. All provisions for stepwise reduction and disease monitoring as described above are to be followed. Additional assessments of plasma ammonia levels, amino acid profiles, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator and as clinically indicated. Learnings from this study will allow for the development of a more standardized protocol for tapering ammonia scavenger medications in future studies of DTX301.

3.2.5 Modification of Dietary Restrictions

Patients with OTC deficiency typically have a personalized dietary regimen, which encompasses protein-restriction adapted to their metabolic and clinical status to optimize the management of their disease.

Subjects will receive a prescribed diet (grams of protein per 24 hours) on Day 0 of the study and this diet will be reviewed at Weeks 6, 12, 24, and 52. Dietary protein intake by the subject between visits and dietary restrictions will be reviewed at each visit (Section 8.7.2 and Table 15-1).

Modification of baseline protein-restricted diet cannot occur at the same time and/or visit as modification of baseline ammonia scavenger therapy. Once medications or diet are adjusted or discontinued, the subject must demonstrate good metabolic control prior to adjustments in the other type of baseline treatment. During periods of adjustment to baseline treatment, subjects will be closely monitored.

Changes to baseline treatment cannot occur while the subject is treated with corticosteroids or within a 2-week period of completing a corticosteroid taper.

At any time after protein-restricted diet has been modified or fully liberalized, reinstitution of restrictions may take place based on the subject's clinical and metabolic status and under evaluation of the investigator. If a subject successfully discontinues alternate pathway medication or diet it may be necessary to restart standard of care therapy during a time of increased metabolic demand, such as

intercurrent viral infection, treatment with steroids, hyperammonemia, or hyperammonemic crisis (Section 3.2.6).

3.2.6 Occurrence of Hyperammonemia and Hyperammonemic Crisis (HAC)

Consistent with the Urea Cycle Disorders Consortium (UCDC), we have defined hyperammonemic crisis (HAC) in this study as an episode of signs and symptoms associated with hyperammonemia (such as frequent vomiting, nausea, headache, lethargy, irritability, combativeness, and somnolence), with documented elevated ammonia levels (≥ 100 µmol/L) and requiring medical intervention [Kent and Holt 2017; Longo and Holt 2017; Diaz 2019].

On-study management of hyperammonemia plasma level or hyperammonemic crises may include, but is not limited to, intravenous hydration, discontinuation of all dietary protein intake, increase in ammonia scavenger therapy, hemodialysis in addition to treatment of underlying inciting illness or event (eg, acute infection, sepsis, bleeding, drug-related, etc.). Comprehensive, internationally accepted guidelines for management of urea cycle disorders are recommended [Häberle 2012; Häberle 2019].

Because there is a wide spectrum of presentation and severity of symptomatic hyperammonemia events, investigator judgment needs to be exercised and each subject's management individualized (Section 3.2.4, Section 8.3.2).

3.2.7 Safety Stopping Criteria

Enrollment will be stopped, and the regulators will be notified if, at any time during the study, any of the following occur:

- Death of a subject following administration of DTX301
- An event with an intensity ≥ Grade 3 (according to the Common Terminology Criteria for Adverse Events (CTCAE) [Section 9.1.3]), but excluding hyperammonemic crises (Section 3.2.6), develops following administration of DTX301
- Occurrence of a hepatic malignancy following administration of DTX301

Following DMC review when a stopping rule is met, if the decision is to restart enrollment, a substantial amendment will need to be approved by the regulatory authority.

Abnormal clinically significant laboratory values (clinical chemistry, plasma ammonia, hematology, urinalysis, and coagulation panel) as assessed by the investigator will be considered AEs. Laboratory values that meet any of the above criteria will result in a pause in enrollment until the DMC reviews the data and provide a recommendation for subsequent study conduct.

Any event that meets the above criteria will be reported immediately (within 24 hours) as outlined in Section 9.1.2.2 and the appropriate page(s) in the electronic case report form (eCRF) must be completed. Enrollment and dosing will be temporarily suspended until the situation can be assessed and risks to the subjects mitigated. The DMC will meet on an ad hoc basis if an investigator reports an AE/SAE that meets any of the study stopping criteria to review and assess the event. If study enrollment is suspended, all subjects who have been enrolled will remain in the study and will continue to be monitored through their completion or withdrawal from the study.

3.2.8 End of Study

Subjects who complete all visits up to and including the Week 52 visit will have completed the study. Subjects who discontinue early will be asked to return for an early withdrawal visit, where all safety assessments should be performed (Table 15-1). Subjects with any ongoing AEs at this visit will continue to be monitored as outlined in Section 9.1.5. After completion of this study, subjects will be asked to enroll in a 4-year extension study to evaluate the long-term (a total of 5 years after dosing) safety and efficacy of DTX301.

4 Subject Selection

Subjects will be assigned to study treatment only if they meet all of the inclusion criteria and none of the exclusion criteria.

4.1 Inclusion Criteria

Each subject must meet all of the following criteria at Screening to be enrolled in this study:

1. Willing and able to provide written informed consent.

2. Males and females ≥ 18 years of age with documented diagnosis of late-onset (defined as first manifestation of signs and symptoms at > 30 days of age) OTC deficiency, confirmed via enzymatic, biochemical, or molecular testing. This may include identification of a pathogenic mutation, via pedigree analysis, liver OTC activity that is < 20% of normal activity, or elevated urinary orotate (> 20 µmol/mmol creatinine) after an allopurinol challenge test [Tuchman 2008a].
3. Documented history of ≥ 1 symptomatic hyperammonemia event with ammonia ≥ 100 µmol/L.
4. Subject's OTC deficiency is stable as evidenced by either a) no clinical symptoms of hyperammonemia OR b) plasma ammonia level < 100 µmol/L within the 4-week period preceding the Screening visit.
5. Subject's plasma ammonia level on Day 1 (predose) is < 100 µmol/L, for patients who historically maintain normal ammonia levels, and the subject is clinically stable; OR subject's plasma ammonia level on Day 1 (predose) is < 200 µmol/L, for patients who historically are not able to fully control ammonia levels with baseline management, and the subject is clinically stable. If the Day 1 (predose) ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results.
6. On ongoing daily stable dose of ammonia scavenger therapy for ≥ 4 weeks.
7. No known allergic reaction to any component of DTX301.
8. Willing and able to comply with study procedures and requirements, including periodic inpatient hospitalizations, frequent blood draws, and urine collections over a 24-hour period.
9. Hematology and coagulation panel results are within the normal range or, if outside the normal range, deemed not clinically significant in the opinion of the investigator.
10. Males and all females of childbearing potential must be willing to use effective contraception at the time of administration of gene transfer and for the 52 weeks following administration of DTX301 to prevent the potential transmission of the AAV vector. For male subjects, appropriate contraceptive methods include the use of a condom with spermicide. For female subjects, appropriate contraceptive methods include the use of a condom with spermicide plus at least 1 of the following:
    a. Oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device;
    b. Use of a diaphragm or cervical/vault cap;

c. Previous female sterilization (surgical bilateral oophorectomy [with or without hysterectomy] or tubal ligation) at least 6 weeks prior to DTX301 administration. In case of an oophorectomy alone, the reproductive status of the subject must have been confirmed by follow-up hormone level assessment.

NOTE: Abstinence is an acceptable form of birth control; however, appropriate contraception must be used if the subject becomes sexually active. Abstinence is defined as sexual inactivity consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea methods are not acceptable methods of contraception. A condom with spermicide is required to be used by all sexually active vasectomized males in the study in order to prevent potential transmission of the vector via seminal fluid.

NOTE: Females of childbearing potential are defined as all females physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during for the duration of the study.

Females are considered post-menopausal and not of childbearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (eg, age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least 6 weeks prior to enrollment. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of childbearing potential.

4.2 Exclusion Criteria

Subjects who meet any of the following criteria at Screening will be excluded from the study:

1. At Screening or Baseline (Day 0), plasma ammonia level ≥ 100 µmol/L for patients who historically maintain normal ammonia levels; OR plasma ammonia level ≥ 200 µmol/L for patients who historically are not able to fully control ammonia levels with baseline management; OR signs and symptoms of hyperammonemia, with documented elevated ammonia level, during the 4-week period preceding Day 0. If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results.
2. Liver transplant, including hepatocyte cell therapy/transplant.

3. History of liver disease as evidenced by any of the following: portal hypertension, ascites, splenomegaly, esophageal varices, hepatic encephalopathy, or a liver biopsy with evidence of stage 3 fibrosis.
4. Significant hepatic inflammation or cirrhosis as evidenced by imaging or any of the following laboratory abnormalities: alanine aminotransferase (ALT) or aspartate aminotransferase > the upper limit of normal (ULN), total bilirubin > 1.5 × ULN, alkaline phosphatase > 2.5 × ULN.
5. Serum creatinine > 2.0 mg/dL.
6. Evidence of active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, documented by current use of antiviral therapy for HBV or HCV or by hepatitis B surface antigen (HBsAg) or HCV RNA positivity. **NOTE:** Subjects with a history of HCV infection must have documentation of 2 negative viral assays by polymerase chain reaction (PCR), collected at least 6 months apart, to be considered negative for HCV. Subjects with a history of HCV infection who test positive for HCV RNA at Screening can be rescreened once, after they have been treated and have documentation of at least 2 negative samples collected at least 6 months apart.
7. History of human immunodeficiency virus (HIV) infection AND any of the following: CD4+ cell count < 350 cells/mm^3, change in antiretroviral therapy regimen within 6 months prior to Day 0, or plasma viral load > 200 copies/mL, documented on 2 separate occasions, as measured by PCR.
8. Active infection (viral or bacterial).
9. Anti-AAV8 neutralizing antibody titer ≥ 1:5.
10. Participation (current or previous) in another gene transfer study.
11. Participation in another investigational medicine study within 3 months of Screening.
12. History of a malignancy for which the subject has received treatment in the past 2 years except for prostate cancer treated with watchful waiting or surgically removed non-melanoma skin cancer.
13. Has a positive serum pregnancy test at Screening (females of childbearing potential only), a positive urine pregnancy test at Baseline (Day 0; females of childbearing potential only), or is nursing.
14. Has any other significant medical condition that the investigator feels would be a risk to the subject or would impede the study.

5 Screening and Randomization Procedures

5.1 Subject Screening

All potential subjects will sign an informed consent form (ICF) before any study procedures or assessments are performed or initiated (Section 11.3). Subjects will have the opportunity to have any questions answered before signing the ICF. All questions raised by the subject must be addressed before the investigator also signs the ICF. A copy of the signed ICF will be given to the subject.

Subjects will be administered [1-^{13}C]sodium acetate orally. Blood samples for determination of ureagenesis will be collected, via an indwelling catheter, before dosing (time 0) and at approximately 0.5, 1, 1.5, 2, 3, and 4 hours after dosing with [1-^{13}C]sodium acetate. During Screening, assessment of rate of ureagenesis may be repeated if discrepant with a subject's clinical status and severity.

Prior to the start of DTX301 infusion, the study site must confirm that the subject's plasma ammonia level on Day 1 (predose) is < 100 µmol/L for patients who historically maintain normal ammonia levels, and the subject is clinically stable; OR the subject's plasma ammonia level on Day 1 (predose) is < 200 µmol/L, for patients who historically are not able to fully control ammonia levels with baseline management, and the subject is clinically stable. If the subject is deemed clinically unstable, dosing with DTX301 will be held, and the subject can be rescreened once the subject is determined to be clinically stable, with repeat of relevant assessments or rescreened at a later date (Section 3.2.1).

Subjects with a history of HCV infection who test positive for HCV RNA at Screening can be rescreened once, after they have been treated and have documentation of at least 2 negative samples collected at least 6 months apart. **NOTE:** To be eligible to participate in the study, subjects must have 2 negative HCV viral assays by PCR at least 6 months apart.

Study sites will maintain documentation of all potential subjects screened for inclusion in the study. If a subject is excluded from the study, the reasons for exclusion will be documented in the subject's source documents and on the screening log.

5.2 Subject Randomization

This is an open-label study; 3 subjects will be enrolled into Cohort 1 and then, sequentially, into cohorts of a minimum of 2 to 3 subjects each, up to Cohort 3. Dosing of additional subjects in expansion of Cohort 3, and initiation of Cohort 4 (Dosing Process Optimization) may occur in parallel (Section 3.1) with at least 1 male subject assigned to each.

6 Study Treatment

6.1 Identity of DTX301

6.1.1 Description of DTX301

DTX301 is a nonreplicating, self-complementary, recombinant AAV8 vector that contains a codon-optimized, wild-type human OTC coding sequence. DTX301 demonstrates thermal stability, which is a general property of AAV and parvoviruses. DTX301 is supplied as a slightly [redacted] DTX301 is a homogeneous, monodisperse solution that is clear and colorless without visible particulates.

6.1.2 Components Used for Manufacturing DTX301

[redacted]

- [redacted]
- [redacted]
- [redacted]

[redacted]. For additional information, please refer to the DTX301 investigator's brochure.

6.2 Management of Clinical Supplies

The study site will be provided supplies required for the infusion of DTX301.

6.2.1 Packaging and Labeling

Each vial of study product provided to study sites will contain 1 mL of DTX301 frozen in 2 mL sterile glass vials with a primary label on the vial. Study product will have secondary packaging with a secondary label. The primary label meets all requirements for blister and small packaging units and will contain a unique identifier. The secondary label will contain required text for all countries participating in the study and will also contain a unique identifier. Secondary labeling will appear in the appropriate language for the country supplied.

6.2.2 Storage of Study Product

6.2.2.1 Storage of DTX301

DTX301 must be stored in a secure freezer at a controlled temperature at or below [redacted]C. The study site is to maintain a daily log documenting the temperature.

6.2.3 Study Product Accountability

The investigator or designated personnel will maintain accurate records of receipt of all study product (DTX301), including dates of receipt. In addition, accurate records will be kept regarding when and how much study product is dispensed and used by each subject in the study. Reasons for departure from the expected dispensing regimen must also be recorded. At the completion of the study, all study product will be reconciled and retained or destroyed according to instructions provided by the sponsor.

6.2.4 Transmission of Infectious Agents

Recombinant AAV vectors are nonreplicative and are not expected to pose a risk of transmission. However, all sexually active subjects must use approved contraception from the time of DTX301 dosing and for 52 weeks following administration (Section 4.1). All subjects enrolled in the study should be encouraged to discuss the use of approved contraception with his or her partner in order to prevent possible transmission of vector via seminal or vaginal fluid. The study product and posttreatment study samples should be handled using standard universal precautions.

6.2.5 Exposure to Radiation

Carbon-13 is a naturally occurring, stable isotope of carbon that emits no radioactivity, has no known adverse biological effects, and is safe to use in children and adults [Koletzko 1997; Tuchman 2008b].

Detailed instructions for dose preparation and administration of [1-^{13}C]sodium acetate are provided in the ureagenesis manual.

6.3 Treatment Schedule and Administration

6.3.1 Administration of [1-^{13}C]Sodium Acetate

Subjects will be administered [redacted] [1-^{13}C]sodium acetate dissolved in 60 mL of water at the time points specified in the Schedule of Events (Table 15-1).

The dose of [1-^{13}C]sodium acetate administered will be calculated using the subject's weight recorded at Screening. Prior to oral administration, calculations will be checked by the study site pharmacist and a member of medical personnel charged with administration of [1-^{13}C]sodium acetate.
The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable in order to receive [1-^{13}C]sodium acetate (Section 8.2.4.1; Section 4.1). If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. If the subject is deemed clinically unstable, administration of [1-^{13}C]sodium acetate will be held until the subject is determined to be clinically stable.

Subjects may experience a temporary, mildly unpleasant salty and/or acidic taste from the sodium acetate component of the compound. The study site must be equipped with emergency resuscitation capabilities. Any event that is considered an AE associated with the administration of [1-^{13}C]sodium acetate (Section 9.1.1) should be recorded in the appropriate page of the eCRF (Section 9.1.2).

Sodium acetate is the sodium salt of acetic acid, a naturally occurring metabolite. Sodium acetate is a commonly used food additive (E 262).

Detailed instructions for dose preparation and administration of [1-^{13}C]sodium acetate are provided in the ureagenesis manual.

6.3.2 Administration of DTX301

Subjects will receive a single, peripheral IV infusion of DTX301, administered by qualified study personnel as designated by the investigator (Table 15-1). The dose will be determined by the cohort and candidate dose (Section 3.1).

The dose of DTX301 to be administered will be calculated using the subject's weight recorded at Screening. The subject's weight will be verified prior to administration of DTX301 to ensure that their current weight is within 10% of their screening weight. **NOTE**: Any subject weighing > 100 kg (> 220 lb) will be dosed as if his or her weight is 100 kg. Prior to infusion, all infusion bag labels will

be checked by the study site pharmacist and a minimum of 2 medical personnel charged with administration of DTX301. **NOTE**: Study product should not be prepared for infusion until the subject's plasma ammonia level on Day 1 is confirmed to be less than 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable (Section 4.1).

The study site must be equipped with emergency resuscitation capabilities. On Day 1, an IV catheter will be inserted into a peripheral vein and flushed with saline.

Detailed instructions for dose preparation and subsequent infusion of DTX301 are provided in the pharmacy manual.

6.3.3 Treatment Compliance

[1-^{13}C]sodium acetate will be administered orally at the study site and observed by qualified personnel. The dose and time of administration will be recorded in the subject's eCRF. Adherence to baseline ammonia scavenger medication regimens and modifications to ammonia scavenger medication regimens (Section 3.2.4) and protein-restricted diet (Section 3.2.5) is also considered treatment compliance.

DTX301 will be administered at the infusion center via a single IV infusion administered by qualified personnel. The dose, start time, stop time, and volume of infusion will be recorded in the subject's eCRF.

6.4 Prior and Concomitant Therapy

Use of all prior and concomitant medications will be recorded in the subject's eCRF. The minimum requirement is that the drug name, the dates of administration, and the reason for use are to be recorded. This will include all prescription drugs, herbal products, vitamins, minerals, and over-the-counter medications. Any changes in concomitant medication use will also be recorded in the subject's eCRF.

6.4.1 Permitted Medications

The use of permitted medications (date, dosage, reason for therapy) will be recorded on the concomitant medication page in the eCRF.

If a subject starts a new medication, including medications to alleviate complications associated with OTC deficiency and herbal supplements, it should be discussed with the investigator.

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given. It is the responsibility of the investigator to ensure that details regarding the medication are recorded in full in the eCRF.

6.4.2 Prohibited Medications

Use of any of the following medications is prohibited, unless the investigator feels these medications are medically indicated. If medically indicated, the use of prohibited medications (date, dosage, reason for therapy) will be recorded on the concomitant medication page in the eCRF:

- Another investigational product to treat OTC deficiency;
- Another gene therapy product; or
- Any other medication currently under investigation.

7 Withdrawal of Subjects From the Study

7.1 Study Withdrawal

Subjects may withdraw from the study at any time and for any reason without prejudice to their future medical care by the investigator or at the study site. Any subject who withdraws consent to participate in the study will be removed from further treatment and/or study observation immediately upon the date of request.

The investigator must record the reason for withdrawal on the relevant page in the eCRF. The reason for withdrawal may include the following:

1. Withdrawal of consent
2. Administrative decision by the investigator or the sponsor
3. Ineligibility
4. Significant protocol deviation
5. Subject noncompliance
6. Adverse event

If a subject is withdrawn due to an AE, the investigator will arrange for the subject to have follow-up visits until the AE has resolved or stabilized (Section 9.1.5).

If a subject requests or decides to withdraw from the study, all efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal, and an early withdrawal visit will be performed.

7.2 Subject Replacement

If a subject withdraws from the study after receiving DTX301, the subject will not be replaced. Subjects who withdraw from the study after signing the ICF, but before receiving DTX301, will be replaced and the replacement subject will be sequentially assigned to treatment with a new subject identification number.

8 Study Assessments and Procedures

8.1 Efficacy Assessments

Planned time points for all efficacy measurements in the study are listed in Table 15-1.

8.1.1 Ureagenesis

The change from baseline in the rate of ureagenesis will be assessed at specified time points (Table 15-1). Planned time points for the collection of blood samples to determine the rate of ureagenesis are provided in Section 8.3.1. Subjects will fast for at least 6 hours, including liquids containing protein, sugar or carbonate, prior to administration of [1-^{13}C]sodium acetate. After dosing, subjects will continue to fast for at least 4 hours. Water is allowed *ad libitum*. During Screening, assessment of rate of ureagenesis may be repeated if discrepant with subject's clinical status and severity.

Details for the preparation and shipment of samples are included in the ureagenesis manual and the laboratory manual.

8.1.2 Plasma Ammonia Area Under the Curve from Time Zero to 24 Hours

The change from baseline in plasma ammonia (AUC_{0-24}) will be assessed at specified time points (Table 15-1). Planned time points for the collection of blood samples to determine plasma ammonia (AUC_{0-24}) are provided in Section 8.3.2. Two samples will be collected at each time point for the plasma ammonia (AUC_{0-24}) determination. One sample will be sent to the local laboratory and 1 sample will be sent to the central laboratory. Details for the preparation and shipment of samples are included in the laboratory manual.

8.2 Safety Assessments

Planned time points for all safety assessments are listed in the Schedules of Events (Table 15-1 and Table 15-2). Safety will be assessed based on AEs, SAEs, complete and targeted physical examination findings, vital sign measurements, ECG results, clinical laboratory assessments (clinical chemistry, plasma ammonia, hematology, urinalysis, and coagulation panel), viral shedding, measurement of neutralizing antibody titer to AAV8, measurement of AAV8 binding antibodies, and measurement of anti-OTC antibodies.

8.2.1 Physical Examination

A complete or targeted physical examination will be performed at the time points specified in the Schedule of Events (Table 15-1).

A complete physical examination will include assessments of the head, eyes, ears, nose, and throat; skin; and the endocrine metabolic, neurological, respiratory, cardiovascular, gastrointestinal, and musculoskeletal systems.

A targeted physical examination will include assessment of the skin and the respiratory, cardiovascular, and gastrointestinal systems.

Physical examination findings will be captured on the appropriate page in the eCRF.

8.2.2 Vital Sign Measurements

Vital sign measurements will be made at the time points specified in the Schedule of Events (Table 15-1). During the study, vital sign measurements are to be collected before any stimulating or anxiety-provoking procedures (eg, phlebotomy). Vital sign measurements will include heart rate, blood pressure (systolic and diastolic), and respiratory rate. Temperature will be included on days that the [1-^{13}C] sodium acetate is administered (Screening, all inpatient visits and Week 20 outpatient visit. Height (at the Screening visit only) and weight will also be recorded.

- On Day 0, weight will be measured to ensure that the weight is within 10% of the screening weight used to calculate the dose of DTX301. Subjects with a confirmed change in weight that is > 10% from Screening should be further evaluated to exclude the presence of an acute condition or illness; if a benign explanation for the weight change is identified and documented, DTX301 can be given and dosed using the weight obtained on Day 0. **NOTE:** Any subject weighing > 100 kg (> 220 lb) will be dosed as if his or her weight is 100 kg.
- Vital signs should be measured with the subject having rested for at least 5 minutes beforehand. It is preferred that the measurement be late with the subject rested, rather than on-time with the subject not sufficiently rested. If the subject is not sufficiently rested, this needs to be stated in the source documents.
- On Day 1, vital signs will be measured at predose, approximately 5 minutes, and 0.5 (±5 minutes), 1 (±5 minutes), 2, 4, 6, and 8 hours (±15 minutes) after the start of DTX301 infusion. Vital signs will also be measured at approximately 22 hours (±1 hour) after the start of infusion, prior to subject discharge.

- It is acceptable for heart rate to be captured from the 12-lead ECG (Section 8.2.3).

Vital sign measurements will be recorded in the appropriate page in the eCRF. The medical monitor should be notified of any clinically significant changes or abnormal value in vital sign measurements (Section 9.1.2).

8.2.3 Electrocardiograms

An ECG will be performed at the time points specified in the Schedule of Events (Table 15-1).

- A single 12-lead ECG will be obtained at Screening, on Day 0 (baseline), at approximately 1 hour (±15 minutes) after the start of infusion on Day 1, and at Week 52, using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and corrected QT intervals.

Twelve-lead ECGs should be measured with the subject having rested for at least 5 minutes beforehand. It is preferred that the measurement be late with the subject rested, rather than on-time with the subject not sufficiently rested. If the subject is not sufficiently rested, this needs to be stated in the source documents. Electrocardiogram results will be recorded in the appropriate page in the eCRF.

8.2.4 Clinical Laboratory Analyses

Laboratory tests, including LFTs and coagulation panel, will be closely monitored throughout the duration of the study. At any point between scheduled visits, additional, unscheduled assessment for LFTs, plasma ammonia, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator.

Investigators will receive flagged notification of any laboratory values that are outside of the normal range. Any abnormal laboratory test results (clinical chemistry, plasma ammonia, hematology, urinalysis, coagulation panel, or other laboratory parameters), including those that worsen from baseline or are felt to be clinically significant in the medical and scientific judgment of the investigator, are to be recorded as AEs or SAEs (Section 9.1.2).

However, any clinically significant safety assessments that are associated with OTC deficiency are **not** to be reported as AEs, **unless** they are judged by the investigator to be more severe than expected for the subject's condition.

All laboratory tests with results that are significantly abnormal during participation in the study should be repeated until the values return to normal or baseline. If such values do not return to normal within a period judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.

Samples of blood, urine, saliva, and stool will be collected for study assessments. Any samples remaining at the end of the study may be stored for up to 15 years and analyzed to better understand the effect of DTX301 on OTC deficiency or other UCDs. The choice to allow retention and future analysis will be optional.

8.2.4.1 Clinical Laboratory Parameters

The clinical laboratory parameters to be measured are listed in Table 8-1. Samples are to be collected at the time points (±5 minutes) specified in the Schedules of Events (Table 15-1 and Table 15-2).

Table 8-1 Clinical Laboratory Parameters

Clinical chemistry[a]:	Sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, glucose, calcium, phosphate, magnesium, albumin, total protein, creatine kinase, bilirubin (total, direct, and indirect), ALT, AST, ALP, gamma-glutamyltransferase, and lactate dehydrogenase
Spot ammonia	Plasma ammonia (STAT sample, local laboratory)[b]
Hematology:	Complete blood count with differential
Urinalysis:	Specific gravity, pH, glucose, protein, blood (by dipstick), ketones (by dipstick), and microscopic examination (if blood or protein is found)
Urine chemistry:	Orotic acid
Coagulation panel:	PT/INR, aPTT
Amino acid panel:	alanine, α-amino-butyric acid, arginine, asparagine, aspartic acid, citrulline, cystine, ethanolamine, glutamic acid, glutamine, glycine, histidine, homocysteine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, and valine
Other[c]:	HBV surface antigen (HBsAg), HCV RNA, HIV

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LFT, liver function test; PT/INR, prothrombin time/international normalized ratio.

a. Through Week 12, one sample for LFTs is collected as part of clinical chemistry and sent to the central laboratory for analysis. A second sample for LFTs only is collected and sent to the local laboratory (STAT sample).

b. Special handling is required for plasma ammonia. At Screening, prior to neuropsychological testing and prior to dosing with [1-^{13}C]sodium acetate, a sample will be collected to confirm the subject's plasma ammonia level. The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable (Section 4.1). If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. If the subject is deemed clinically unstable, the assessments will be held until the subject is determined to be clinically stable. (Table 15-1). The study site may use the local laboratory sample from time 0 (prior to neuropsychological testing on Day 0 and at Week 52), and time 24 hour (prior to [1-^{13}C]sodium acetate administration on Day 1 and at Weeks 6, 12, 24, and 52) from the plasma ammonia AUC_{0-24} to confirm the subject's plasma ammonia (Section 8.3.2).

c. To be collected at Screening only.

Details for the preparation and shipment of samples will be provided by the central or specialty laboratory. Reference ranges for all parameters will be provided to the study site by the central or specialty laboratory.

A laboratory parameter may be repeated if there is any concern about the values obtained.

At any time after initiation of a prophylactic corticosteroid regimen, additional assessments of plasma ammonia levels, amino acid profiles, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator and as clinically indicated.

If additional nonprotocol-specified laboratory assessments are performed at the study site's local laboratory and result in a change in subject management or the results are considered clinically

significant by the investigator (eg, SAE, AE, or dose modification), the results must be captured and sent to the sponsor along with other study data as defined in Section 9.1.2.

8.2.4.1.1 Elevation of Liver Function Tests

In clinical studies with AAV-mediated gene transfer, a transient rise in liver aminotransferases and concurrent decline in transgene expression has been observed [Manno 2006; Nathwani 2011a; Nathwani 2014]. This has been hypothesized to be due to the activation of capsid-specific cytotoxic T lymphocytes and destruction of transduced liver cells [Mingozzi 2007]. However, in mice, T-cells activated against AAV capsid were not able to target and eliminate transduced hepatocytes [Wang 2007; Li C 2007; Li H 2007; Siders 2009] unless the transduced hepatocytes co-expressed the wild-type AAV capsid protein [Li C 2007].

The inability to reproduce the observed effects in animal models has made it difficult to assess the validity of the hypothesis or to develop strategies to overcome or minimize aminotransferase elevations. Despite a lack of clear resolution that activation of cytotoxic T lymphocytes leads to a reduction in transgene expression from hepatocytes, appropriate precautions have been incorporated into this study. The use of oral steroids is allowed if a subject experiences elevations in liver aminotransferases.

Liver function tests will be assessed as part of clinical chemistry (Section 8.2.4.1) at the time points specified in the Schedule of Events (Table 15-1 and Table 15-2). Liver function tests will be assessed at a central and local (STAT) laboratory approximately every 4 days through Week 12, which will allow for a rapid detection of any elevations following administration of DTX301. If a subject experiences an increase in liver function tests following treatment with DTX301, the investigator should consider the initiation of steroid treatment as outlined in Section 8.2.4.1.2, and taking into account dosing Cohort (Cohort 1-3). The event should be recorded as an AE or SAE, as defined in Section 9.1.2, on the appropriate pages in the eCRF if it is felt to be clinically significant in the medical and scientific judgment of the investigator.

Cohort 4 (Dosing Process Optimization) will utilize an alternative regimen of corticosteroids, aiming at prophylaxis of AAV-mediated, transient rises in liver aminotransferases as outlined in Section 8.2.4.1.2. The subject must be assessed as clinically and metabolically stable to initiate the prophylactic corticosteroid regimen.

8.2.4.1.2 Treatment for Possible Vector-Induced Hepatitis

The investigator, in conjunction with the Ultragenyx Pharmaceutical Inc. medical lead will consider starting oral steroid treatment, per protocol, for possible vector-induced hepatitis when a subject's ALT **is greater than the ULN** and is considered by the investigator to be related to treatment with DTX301. The subject must be assessed as clinically and metabolically stable, and intercurrent illnesses (eg, viral infection) or concomitant medications known to affect transaminases, be excluded.

Repeat liver function testing for confirmation of results should be considered to inform the use of steroid treatment. If repeat testing is deemed necessary, every effort should be made to repeat the testing within 24 hours from receipt of test results indicating elevated ALT.

At any point between scheduled visits, additional, unscheduled assessment for LFTs, plasma ammonia, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator.

Changes to baseline treatment (ie, ammonia scavenger medications and protein-restricted diet) cannot occur while the subject is treated with corticosteroids or within a 2-week period of completing a corticosteroid taper.

Based on available evidence, it is expected that vector-induced hepatitis will be self-limiting. Therefore, oral prednisone (or prednisolone) will be used per the American Association for the Study of Liver Disease guidelines, with a slight modification in **Cohorts 1 to 3**:

- Week 1: prednisone 60 mg/day
- Week 2: prednisone 40 mg/day
- Week 3 and Week 4: prednisone 30 mg/day
- Week 5 and thereafter: prednisone will be tapered by 5 mg/week until liver enzymes return to baseline levels. The use of prednisone will be recorded on the appropriate page in the eCRF.

Cohort 4 (Dosing Process Optimization) will utilize an alternative regimen of corticosteroids, aiming at prophylaxis of vector-induced hepatitis. Therefore, oral prednisone (or prednisolone) will be initiated before dosing with DTX301, sustained for 4 weeks, followed by tapering as described below. Prophylactic corticosteroid regimens are in use for gene transfer therapies [Audentes 2017 Clinicaltrials.gov identifier: NCT03223194; Abeona Therapeutics 2016 ClinicalTrials.gov identifier: NCT02716246].

- At least 5 days prior to dosing: prednisone 60 mg/day
- Study Day 1 (Week 1) through Week 4: prednisone 60 mg/day
- Study Week 5 through Week 8, tapering every 5 days:

Taper Dose	**Duration**
40 mg/ day	5 days
30 mg/day	5 days
20 mg/day	5 days
10 mg/day	5 days
5 mg/day	5 days
5 mg every other day	5 days

At any time after initiation of prophylactic corticosteroid regimen, additional assessments of plasma ammonia levels, amino acid profiles, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator and as clinically indicated.

Utilization of prednisone (or prednisolone) after dosing and after completion of the prophylactic regimen, remains available to all subjects when a subject's ALT is greater than the ULN and is considered by the investigator to be related to treatment with DTX301.The use of prednisone (or prednisolone) will be recorded on the appropriate page in the eCRF.

8.2.5 Other Laboratory Parameters

Where applicable, details for the preparation and shipment of samples are included in the laboratory manual.

8.2.5.1 Neutralizing Antibodies to Adeno-Associated Virus Serotype 8

Samples for neutralizing antibodies to AAV8 will be collected at the time points specified in the Schedule of Events (Table 15-1) to monitor for a humoral immune response to AAV8. The assay will be performed using a research method (a cell-based assay).

8.2.5.2 Adeno-Associated Virus Serotype 8 Binding Antibody Immunoglobulin G Assay

Samples for the AAV8 binding antibody immunoglobulin G (IgG) assay will be collected at the time points specified in the Schedule of Events (Table 15-1) to monitor for circulating anti-AAV8 antibodies. The assay will be performed using a research method (enzyme-linked immunosorbent assay [ELISA]).

8.2.5.3 Anti-Ornithine Transcarbamylase Antibody Assay

Samples for the anti-OTC antibody assay will be collected at the time points specified in the Schedule of Events (Table 15-1) to monitor for circulating anti-OTC antibodies. The assay will be performed using a research method (ELISA).

8.2.5.4 Viral Shedding

Saliva, urine, and stool will be collected at the time points specified in the Schedules of Events (Table 15-1 and Table 15-2) to monitor for the presence of shed virus. The presence of DTX301 will be determined [redacted]. Subjects will be given an appropriate container to collect a stool sample at home. Samples for shedding analysis will be collected until the results are negative on 3 consecutive occasions for each matrix.

8.3 Pharmacokinetics and Pharmacodynamic Assessments

8.3.1 Ureagenesis

The conversion of the stable isotope [1-^{13}C]sodium acetate to [^{13}C]urea will be determined by gas chromatography mass spectrometry [Tuchman 2008b]. Blood samples will be collected in precooled heparinized tubes before dosing (time 0) and at approximately 0.5, 1, 1.5, 2, 3, and 4 hours (±5 minutes) after [1-^{13}C]sodium acetate is administered orally at Screening, Baseline (Day 1), and over time to Week 52 after administration of DTX301 (Table 15-1). [redacted]. During Screening, assessment of rate of ureagenesis may be repeated if discrepant with subject's clinical status and severity. Blood samples will be immediately centrifuged to separate the plasma, which will be stored frozen at –70°C until shipped for analysis. Details for the preparation and shipment of samples are included in the ureagenesis manual and the laboratory manual.

8.3.2 Plasma Ammonia Area Under the Curve from Time Zero to 24 Hours

The AUC_{0-24} of plasma ammonia will be determined at Baseline (Day 0) and over time to Week 52 after administration of DTX301 (Table 15-1). Two samples will be collected at time 0 and at approximately 2, 4, 8, 12, 16, 20, and 24 hours (±5 minutes). One sample will be sent to the local laboratory (STAT sample) and 1 sample will be sent to the central laboratory. If possible, ad hoc AUC_{0-24} plasma ammonia should be considered if a subject experiences symptomology deemed by the investigator to meet the definition of an HAC (Section 3.2.6), driven by the underlying disease or at a time of increased metabolic demand, such as intercurrent viral infection, or treatment with corticosteroids, and warranting inpatient monitoring based on investigator discretion.

The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable (Section 4.1) prior to administering the neuropsychological tests or [1-^{13}C]sodium acetate and DTX301 (Day 1 only) (Table 15-1). If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. If the subject is deemed clinically unstable, the assessments will be held until the subject is determined to be clinically stable. The study site may use the time 0 and time 24 hour plasma ammonia samples to confirm the subject's plasma ammonia. The samples should be sent to the local laboratory (STAT sample) and results available prior to initiating the neuropsychological tests or administering [1-^{13}C]sodium acetate and DTX301 (Day 1 only).

Details for the preparation and shipment of samples are included in the laboratory manual.

8.3.3 Orotic Acid Excretion

The excretion of orotic acid will be determined over a 24-hour period. Urine samples will be collected approximately every 6 hours (× 4) at Baseline (Day 0) and over time to Week 52 after administration of DTX301 (Table 15-1). Details for the preparation and shipment of samples are included in the laboratory manual.

8.4 Quality-of-Life Assessment

8.4.1 PROMIS Questionnaire

Subjects will be asked to complete the Patient-Reported Outcomes Measurement Information System (PROMIS) questionnaire (Appendix 15.2.1) on Day 0 (predose) and then at Weeks 6, 12, 24, and 52 during the study (Table 15-1). **NOTE:** The PROMIS questionnaire should be completed prior to the neuropsychological tests on Day 0 and at Week 52.

Patient-reported outcomes will be assessed by components of the PROMIS questionnaire, an instrument developed in collaboration with National Institutes of Health, which has been designed, calibrated, and validated using a large sample size of participants with chronic diseases for use in clinical care and research. PROMIS measures may be used as endpoints in clinical studies to assess the effectiveness of treatment. The UCD Consortium also uses the PROMIS questionnaire for the evaluation of QoL in their Longitudinal Study participants.

Domains measured by PROMIS include physical, mental, and social well-being; versions of the PROMIS instrument include ones specific for adult and pediatric populations as well as for parent-proxy reporting for pediatric patients. The adult versions for the following self-reported domains will be included in this study [Reeve 2007; PROMIS 2009]: Cognitive Function, Anxiety, Depression, and Emotional/Behavioral Dyscontrol.

8.5 Neuropsychological Tests

All neuropsychological tests will be administered to English speaking subjects (at a minimum) in a nonfasted state. All neuropsychological tests are to be administered by appropriately qualified individuals as determined by sponsor or representative. The subject's plasma ammonia level should be < 100 µmol/L (Section 8.2.4.1) or within the range of historical ammonia levels obtained when the subject was clinically stable (Section 4.1) in order to perform the neuropsychological tests. If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. The study site may use the time 0 (T 0) plasma ammonia sample (from

AUC_{0-24} plasma ammonia) analyzed by the local laboratory (STAT sample) to determine the subject's plasma ammonia level. The result must be available prior to initiating the tests. If the subject is deemed clinically unstable, the neuropsychological tests will be held until the subject is determined to be clinically stable. Subjects may request pauses to rest during administration of the neuropsychological test battery, but the entire battery should be completed in 1 session. Additional details are provided in the neuropsychological section of the manual of operations.

The neuropsychological assessments have been selected in close consultation with Dr. Susan Waisbren and are based on assessments where OTC participants in the UCD Consortium Longitudinal Study have shown cognitive deficits thus far (refer to the neuropsychological section of the manual of operations for further details). The most informative neuropsychological tests, as determined by these Phase 1/2 study data, may be included in further clinical studies with DTX301.

Neuropsychological deficits, especially in memory and executive functioning, are considered to be important aspects of the OTC deficiency condition and have widespread effects on daily functioning and relationships. Improving or normalizing plasma ammonia and other blood biomarkers (glutamate and glutamine) is hypothesized to potentially improve neuropsychological functioning. Therefore, it is important to establish neuropsychological functioning prior to dosing with DTX301 as well as evaluate the trajectory of this functioning longitudinally, ideally over several years. During the 4-year extension study, subjects will take this same neuropsychological battery of tests every 2 years to assess changes over time in motor, executive, and memory function.

8.5.1 Intelligence

Subjects will be asked to complete the Vocabulary and Matrix Reasoning subtests from the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II) at Baseline only (Table 15-1). The WASI-II provides general measure of cognitive function in subjects aged 6 to 89 years. The Vocabulary and Matrix Reasoning subtests provides an accurate, validated assessment of IQ that is highly correlated (r=0.86) with results from the Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV) [Wechsler 2011]. The estimated time needed for completion of the WASI-II is 30 minutes.

8.5.2 Motor Function

8.5.2.1 [redacted]

[redacted]

8.5.3 Executive Function

8.5.3.1 [redacted]

[redacted]

8.5.4 Memory

8.5.4.1 [redacted]

[redacted]

[redacted]

8.6 Genotyping

8.6.1 Ornithine Transcarbamylase Genotyping

At Baseline, subjects will be asked to provide a single whole-blood sample for OTC genotyping. The objective of this research is to provide a background understanding to the etiology of the subject's OTC deficiency and to investigate any relationship between genetic factors and the subject's response to DTX301. The results do not need to be available prior to dosing with DTX301.

8.7 Other Assessments

8.7.1 Demographic, Medical, and Ornithine Transcarbamylase Deficiency History Assessments

As allowed by local laws and regulations, the following demographic data may be captured on the appropriate page in the eCRF: date of birth, sex, race, and ethnicity.

Medical, medication, and OTC deficiency medical history will be assessed as related to the eligibility criteria listed in Section 4.1 and Section 4.2.

8.7.2 Ammonia Scavenger Use and Dietary Protein Intake

The use of ammonia scavenger therapy and dietary protein intake will be reviewed and recorded at each visit (Table 15-1).

Changes to baseline treatment cannot occur until there is evidence of transgene expression reflected by continued evidence of metabolic stability with ammonia levels in the normal range or improved ammonia control if subjects have a history of ammonia levels above the upper limit of normal and supported by improvement in clinical signs and symptoms. The subject must be clinically stable and under good metabolic control before changes can be initiated or progressed. The risks of making adjustments to baseline treatment on their own, without express guidance from the site, will be reinforced with the subject at site visits.

Changes to baseline treatment cannot occur while the subject is treated with corticosteroids or within a 2-week period of completing a corticosteroid taper.

Tapering or discontinuation of ammonia scavenger therapy cannot occur at the same time and/or visit as modification of protein-restricted diet. At any time after modification and/or discontinuation of ammonia scavenger therapy, reinstitution of therapy may take place based on the subject's clinical and metabolic status (elevated ammonia levels, or signs and symptoms consistent with hyperammonemia), and under evaluation of the investigator.

Subjects will receive a prescribed diet (grams of protein per 24 hours) on Day 0 of the study and this diet will be reviewed at Weeks 6, 12, 24 and 52. Dietary protein intake by the subject between visits and dietary restrictions will be reviewed at each visit (Table 15-1) and recorded in the source documentation. **Modification of baseline protein-restricted diet cannot occur at the same time and/or visit as modification of baseline ammonia scavenger therapy.** At any time after protein-restricted diet has been modified or fully liberalized, reinstitution of restrictions may take place based on the subject's clinical and metabolic status and under evaluation of the investigator.

Once medications or diet are adjusted or discontinued, the subject must demonstrate good metabolic control prior to adjustments in the other type of baseline treatment.

9 Safety Monitoring and Reporting

9.1 Adverse Events and Serious Adverse Events

Adverse events will be assessed from the time the subject signs the ICF through the end of study/early withdrawal visit.

At every study visit, subjects will be asked a standard nonleading question to elicit any medically related changes in their well-being.

In addition to subject observations, AEs identified from any study data (eg, laboratory values, physical examination findings, ECG changes) or identified from review of other documents (eg, subject diaries) that are relevant to subject safety will be documented on the AE page in the eCRF.

9.1.1 Definitions

9.1.1.1 Adverse Events

The investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study product or their clinical significance.

An AE is defined as any untoward medical occurrence in a subject enrolled into this study regardless of its causal relationship to study product. Subjects will be instructed to contact the investigator at any time after the subject signs the informed consent if any signs or symptoms develop.

A treatment-emergent AE is defined as any event not present before exposure to study product or any event already present that worsens in either intensity or frequency after exposure to study product.

9.1.1.2 Serious Adverse Events

An SAE is defined as any event that:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization.
  - **NOTE:** Hospitalization due to hyperammonemic crisis (HAC [Section 3.2.6]) will be considered an SAE.
  - **NOTE**: Nonemergent hospitalizations for subject monitoring in the setting of steroid administration for vector-induced hepatitis (Section 8.2.4.1.2) will not be considered an SAE.
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect

Additionally, important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

9.1.2 Safety Reporting

9.1.2.1 Adverse Events

All AEs reported or observed during the study will be recorded on the AE page in the eCRF. Information to be collected includes drug treatment, dose, event term, time of onset, investigator-specified assessment of severity and relationship to study product, corticosteroid

regimen, [1-^{13}C] sodium acetate, OTC deficiency, or hyperammonemia, time of resolution of the event, seriousness, any required treatment or evaluations, and outcome. Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution. The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code all AEs.

Any medical condition that is present at the time that the subject is screened but does not deteriorate should not be reported as an AE. However, if the condition deteriorates at any time during the study, it should be recorded as an AE.

9.1.2.2 Serious Adverse Events

Any AE that meets SAE criteria (Section 9.1.1.2) or any of the safety stopping criteria (Section 3.2.7) must be reported by the study site to PPD Pharmacovigilance (PVG) Department immediately (ie, within 24 hours) after the time study site personnel first learn about the event. The study site should record all SAE information in the SAE eCRF and submit the report via the electronic data capture (EDC) system. An event meeting any of the safety stopping criteria should only be reported as an SAE in the EDC system if it meets SAE criteria (Section 9.1.1.2).

In the event of any fatal or life-threatening SAE, the investigator must immediately inform PPD PVG by telephone (Table 9-1) and report the SAE in the EDC system. If, for any reason, it is not possible to report the SAE in the EDC system (eg, the EDC system is unavailable), the study site should record the SAE on the paper SAE Reporting Form and fax it to PPD PVG (Table 9-1). As soon as it is possible, any SAE reported via fax must be entered into the EDC system.

Table 9-1 PPD PVG Contact Information for SAE Reporting

Region	Contact Information
Rest of World	**Safety hotline**: [redacted]
	Safety fax[a]: [redacted]
North America	**Safety hotline**: [redacted]
	Safety fax[a]: [redacted]

Abbreviations: PVG, pharmacovigilance; SAE, serious adverse event.

a. This is the preferred fax number for all regions. The toll-free fax number for North America can be provided upon request.

9.1.2.2.1 Expedited Reporting

The sponsor is responsible for reporting serious, unexpected, suspected adverse drug reactions (SUSARs) involving the study product(s) to all regulatory authorities and participating investigators in accordance with International Council for Harmonisation (ICH) guidelines and/or local regulatory requirements, as applicable. It is the responsibility of the investigator or designee to promptly notify the local institutional review board (IRB)/independent ethics committee (IEC)/institutional biosafety committee (IBC) of all SUSARs involving risk to human subjects.

As there is no prior clinical experience with DTX301, reference safety information for assessing whether an AE is a SUSAR is currently not available. Therefore, any SAE considered related to DTX301 will be considered a SUSAR and reported immediately as detailed in Section 9.1.2. The SAE should be treated with appropriate supportive and medical care deemed necessary for the well-being of the subject.

9.1.3 Assessment of Severity/Toxicity

The severity/toxicity, or intensity, of an AE refers to the extent to which an AE affects the subject's daily activities. The intensity of the AE will be rated as Grade 1, 2, 3, 4, or 5 using the most current version of the National Cancer Institute (NCI) CTCAE [NCI CTCAE 2018]. The CTCAE provides descriptive terminology that can be used to standardize AE reporting. A severity/toxicity grade is provided for each AE term that is grouped by the highest level of MedDRA classification [NCI CTCAE 2018]. Specific symptoms and medical conditions have a clinical description for each level of severity/toxicity. In the event that an AE occurs during the study that is not captured by the CTCAE, the AE should be graded according to the general guidelines outlined in Table 9-2.

Table 9-2 General Guidelines for Grading Events not Captured by the CTCAE

Grade	Criteria
Grade 1:	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2:	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[a].
Grade 3:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b].
Grade 4:	Life-threatening consequences; urgent intervention indicated.
Grade 5:	Death related to AE.

Abbreviations: ADL, activities of daily living; AE, adverse event.

a. Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

b. Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Changes in the severity/toxicity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent do not require documentation of onset and duration of each episode.

9.1.4 Assessment of Causality

The investigator's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

The relationship or association of the study product in causing or contributing to the AE will be characterized using the following classification and criteria:

Unrelated: This relationship suggests that there is no association between the study product and the reported event.

Possible: This relationship suggests that treatment with the study product is causing or contributing to the AE; ie, the event follows a reasonable temporal sequence from the time of the study product administration or follows a known response pattern to the study product but could also be produced by other factors.

Probable: This relationship suggests that a reasonable temporal sequence of the event with the study product administration exists and, based upon the known pharmacological action of the study product, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the investigator's clinical experience, the association of the event with the study product seems likely. The event disappears or decreases on cessation or reduction of the dose of study product.

Definite: This relationship suggests that a definite causal relationship exists between the study product administration and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event. The event reappears or worsens if the study product is re-administered.

The relationship or association of corticosteroid regimen, [1-^{13}C] sodium acetate, OTC deficiency, or hyperammonemia in causing or contributing to the AE will also be characterized using the classification terms above.

9.1.5 Follow-Up of Subjects Reporting Adverse Events

All AEs must be reported in detail on the appropriate page in the eCRF and followed to satisfactory resolution, until the investigator deems the event to be chronic or not clinically significant, or until the subject is considered to be stable.

9.2 Procedures for Handling Special Situations

9.2.1 Pregnancy

A serum pregnancy test will be performed on all female study subjects of childbearing potential during Screening. A urine pregnancy test will be performed on all female study subjects of childbearing potential at each visit specified in the Schedule of Events (Table 15-1).

Pregnancy is not regarded as an AE unless there is a suspicion that a study drug may have interfered with the effectiveness of a contraceptive medication. Any pregnancy in a female study subject that occurs during study participation must be reported using the paper Pregnancy Report Form and fax it to PPD PVG (Table 9-1). To ensure subject safety, each pregnancy in a female study subject must be reported to PPD PVG Department (contact information in Section 9.1.2.2) within 2 weeks of learning of its occurrence.

The pregnancy in a female study subject must be followed up to determine outcome (including spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) and status of mother and child, even if the subject discontinued from the study. Pregnancy complications and elective terminations for medical reasons should not be reported as an AE or SAE. Spontaneous miscarriages must be reported as an SAE. All neonatal deaths that occur within 28 days of birth should be reported as an SAE.

Any SAE occurring in association with a pregnancy in a female study subject brought to the investigator's attention after the subject has completed the study and considered by the investigator as possibly related to the study product, must be promptly reported to Ultragenyx Pharmaceutical.

9.2.2 Treatment Noncompliance

9.2.2.1 Overdose Management

An overdose is any dose of study product given to or taken by a subject that intentionally or unintentionally exceeds the dose, based on body weight (kg), described in Section 3.1. Overdoses without signs or symptoms do not need to be recorded as AEs; in case of any AEs associated with the overdose, these should be reported on the relevant AE or SAE section in the eCRF. The actual dose infused will be recorded on the appropriate page in the eCRF.

There is no treatment for overdose. All subjects will be closely monitored at the time of infusion for any adverse effects and supportive care will be administered at the discretion of the investigator, as needed, should an overdose be suspected.

9.2.2.2 Medication Errors

A medication error is defined as a mistake made in prescribing, dispensing, administration, or use of the study product. The treatment will be open-label and is to be administered by trained medical personnel at the study site.

9.3 Data Monitoring Committee

An independent DMC will be responsible for monitoring safety data from the study. The DMC will meet after all subjects in Cohort 1, all subjects in Cohort 2, and the initial 3 subjects in Cohort 3 have completed Week 12 of the study to review the safety data and to provide their recommendation for progressing to the next dosing cohort, or expansion of a dosing cohort. There will be no intra-cohort dose escalations. The DMC will also hold a meeting at completion of Week 12 for the first 3 subjects dosed in Cohort 4, and at the completion of Week 52 for all subjects. Additional DMC meetings may take place, as needed.

The DMC will meet on an ad hoc basis if an investigator reports an AE/SAE that meets any of the safety stopping criteria (Section 3.2.7). The DMC may, at any time, recommend modifying or pausing enrollment due to safety concerns based on these periodic data reviews. Following DMC review when a stopping rule is met, if the decision is to restart enrollment, a substantial amendment will need to be approved by the regulatory authority. The full scope of each review will be outlined in the DMC charter. The DMC will comprise at least either 3 independent medical professionals or 2 independent medical professionals and an independent biostatistician who are qualified to review the data and provide recommendations for progressing to the next dosing level and progressing to the next dosing cohort and cohort expansion. The DMC charter details the members' roles and responsibilities as part of the DMC as well as the process for each data review (ad hoc or scheduled).

10 Statistical and Analytical Plan

A statistical analysis plan (SAP) will be written and will provide a detailed description of the statistical methods and expand on the details provided in this protocol. Additional analyses may be added.

10.1 Dose-Finding Algorithm and Process

Dose finding for DTX301 will be accomplished through a CRM algorithm as described in Section 10.5.1. The operational process is as follows:

- Data sources and methodologies used to determine the parameter values of the prior distributions of the toxicity model
- Data elements and formats required in order to conduct modeling needed for producing dosing recommendation for the next dose

- Validation process of the modeling results and format and data to be provided to the DMC for dosing decisions

10.2 Primary Endpoints

The primary endpoint is the incidence of AEs, treatment-emergent AEs, and SAEs (Section 9.1).

10.3 Secondary Endpoints

The secondary endpoints are:

- The change from baseline in the rate of ureagenesis (Section 8.1.1)
- The change from baseline in $AUC_{0\text{-}24}$ for plasma ammonia (Section 8.1.2)

10.4 Exploratory Endpoints

The exploratory endpoints are:

- The number of hyperammonemic crises (HAC) (Section 8.2.4.1)
- The change from baseline in urinary orotic acid secretion (Section 8.3.3)
- The change from baseline in serum glutamine and glutamate (Section 8.2.4.1)
- Changes in responses to neuropsychological tests (Section 8.4)
- Responses to the PROMIS questionnaire (Section 8.4.1)
- Use of ammonia scavenger therapy (Section 8.7.2)
- Change in dietary protein intake (Section 8.7.2)
- The development of neutralizing antibodies to AAV8 (Section 8.2.5.1)
- The development of anti-AAV8 binding antibodies (Section 8.2.5.2)
- The development of anti-OTC antibodies (Section 8.2.5.3)

10.5 Statistical Analysis Methodology

Dose-finding modeling will be conducted through specialized software that has been validated by PPD. SAS® software (SAS Institute, Inc., Cary, North Carolina, United States) Version 9.2 or later

will be used for general data manipulation and statistical analyses. Continuous variables will be summarized using the mean, the standard deviation, median, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages. Data will be listed in data listings.

Details of the statistical analyses, methods, and data conventions are described in the SAP.

10.5.1 Determination of the Optimal Biological Dose

The CRM uses the Bayesian method to model the probability of experiencing a DLT for each given dose in order to determine the next dose. A DLT is defined as any AE/SAE ≥ Grade 3 that is considered related to DTX301 by the investigator [NCI CTCAE 2018]. [redacted] The OBD is defined as the highest dose where the predicted probability of experiencing a DLT is less than the target toxicity level. [redacted]

A high-level overview of the decision criteria to be used during dose finding are presented in Figure 10-1. Subjects (n=3) in the first cohort (Cohort 1) will be assigned to Dose 1. [redacted] Dose finding will continue in this manner until one of the following conditions is met:

1. The MTD is reached. [redacted]
2. The OBD is found. [redacted]
3. All of the dosing cohorts have been enrolled.

[redacted]

Possible dosing paths during dose [redacted] The protocol will also allow for adding an intermediate dose during either escalation or de-escalation, relative to the candidate doses outlined in

[redacted]

10.5.2 Efficacy Analysis

10.5.2.1 Ureagenesis

The change from baseline in the rate of ureagenesis (presented in µmol/kg/hr and the relative percentage to normal healthy adults [300 µmol/kg/hr]) will be determined for all subjects at Weeks 6, 12, 20, 24, and 52 (Table 15-1). During Screening, assessment of rate of ureagenesis may be repeated if discrepant with subject's clinical status and severity. Full details of the analysis will be provided in the SAP.

10.5.2.2 Plasma Ammonia Area Under the Curve from Time Zero to 24 Hours

The change from baseline in the plasma ammonia area under the curve (AUC_{0-24}) and the time-normalized plasma ammonia, defined as plasma ammonia AUC_{0-24} divided by actual hours from zero to 24 hours, after IV administration of DTX301 will be determined for all subjects at Weeks 6, 12, 24, and 52 (Table 15-1). Full details of the analysis will be provided in the SAP.

10.5.3 Safety Analyses

All subjects who receive DTX301 will be included in the safety analysis.

10.5.3.1 Adverse Events

All statistical analyses of safety outcomes will be descriptive. The incidence of AEs and treatment-emergent AEs will be summarized for each dosing cohort by severity and relationship to study product. Serious AEs will be presented for each dosing cohort by relationship to study product. Summary tables will present incidence estimates and individual event rates by system organ class as well as within each system organ class. Subjects experiencing an event more than once with varying severity will be counted only once, applying only the maximum severity observed within each system organ class and preferred term. For incidence of relationship to study product, subjects will be counted only once, in the category of the strongest relationship to study product within each system organ class and preferred term.

10.5.3.2 Physical Examination Findings

Complete and targeted physical examination findings will be summarized by visit and dosing cohort.

10.5.3.3 Vital Sign Measurements

Vital sign measurements (heart rate, blood pressure [systolic and diastolic], and respiratory rate) will be summarized over time in terms of absolute values and changes from baseline by visit and dosing cohort. Height and weight will be summarized.

10.5.3.4 Electrocardiogram Results

Electrocardiogram data will be summarized by visit and dosing cohort. Each ECG will be classified as “abnormal” or “normal,” and the relevance of the abnormality will be summarized as “clinically significant” or “not clinically significant.”

10.5.3.5 Clinical Laboratory Assessment Results

For all laboratory assessments with continuous results, absolute values and changes from baseline will be summarized by visit and dosing cohort. For laboratory tests with categorical results, shifts from baseline will be summarized by visit and dosing cohort. Chemistry values from both the central laboratory and subjects' local laboratories will be analyzed.

10.5.3.6 Other Laboratory Results

Neutralizing antibodies (AAV8) and AAV8-binding antibody IgG assay results will be listed by time point and dosing cohort. Anti-OTC antibody assay and viral shedding results will be listed by time point and dosing cohort.

10.5.4 Neuropsychological Tests

Changes in responses to the PROMIS mental health measurements and [redacted] (Section 8.4) will be summarized by visit and dosing cohort as outlined in the SAP. Results of the other neuropsychological tests will be listed by visit and dosing cohort.

10.5.5 Quality-of-Life Assessment

Associations between QoL and dose will be undertaken using tabular summaries and appropriate statistical methods as outlined in the SAP.

10.5.6 Other Analyses

Summary statistical analyses will be provided for demographics, medical history, OTC deficiency medical history, prior and concomitant medications, use of ammonia scavengers, and dietary protein intake. A summary of subject disposition will be prepared.

10.5.7 Interim Analysis

An interim analysis may be conducted when 12 weeks of data are available for all subjects from at least 2 dosing cohorts. Results and their dissemination will be at the sponsor's discretion. A detailed plan for the analysis of the safety and efficacy data will be presented in the SAP.

10.6 Data Quality Assurance

Study sites will maintain source documentation and enter subject data in the eCRF as accurately as possible and will rapidly respond to any reported discrepancies. The eCRFs are accessed through Medidata Rave® (New York, New York, United States). This EDC system is validated and compliant with US Title 21 Code of Federal Regulations (CFR) Part A1. Each person involved with the study will have an individual user name and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records, as well as the time and date of any modifications. A quality review of the data will be performed by the study site with additional reviews by the clinical monitor.

Each eCRF is presented as an electronic copy, allowing data entry by study site personnel, who can add and edit data, add new subjects, identify and resolve discrepancies, and view records. This system provides immediate direct data transfer to the database, as well as immediate detection of discrepancies, enabling study site coordinators to resolve and manage discrepancies in a timely manner.

Paper copies of the eCRFs and other database reports may be printed and signed by the investigator. This system provides study site personnel, monitors, and reviewers with access to hardcopy audits, discrepancy reviews, and investigator comment information.

After all queries have been resolved, the SAP is approved and signed, and any summary/analysis populations are approved, the database will be locked. All summary and analysis of the data will be performed using SAS software Version 9.2 or later.

10.6.1 Data Management

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include diary cards, laboratory reports, ECG strips, and other materials.

The sponsor (or sponsor designee) will supply the eCRF. Study personnel must have documented training in the use of the EDC system before the system can be authorized.

All eCRF information is to be completed. If an item is not available or is not applicable, this fact should be indicated.

Investigative study site personnel will enter subject data into the EDC system. The analysis data sets will be a combination of these data and data from other sources (eg, laboratory data). All entries and changes to the data in the eCRF will be recorded electronically with an audit trail specifying the date and time of entry or change and the name of the authorized person making the entry or change. The investigator will answer all queries issued, if applicable. Data queries and query correspondence will be included in the audit trail.

Clinical data management will be performed in accordance with applicable sponsor (or sponsor designee) standards and data cleaning procedures to ensure the integrity of the data (eg, removing errors and inconsistencies in the data). Adverse events and concomitant medication terms will be coded using MedDRA and WHO Drug dictionary terminology, respectively.

After database lock, each study site will receive an electronic copy of their study site-specific eCRF data as entered into the EDC system for the study, including full discrepancy and audit history. Additionally, an electronic copy of all of the study site's data from the study will be created and sent to the sponsor for storage. The sponsor (or sponsor designee) will maintain a duplicate electronic copy for their records. In all cases, subject initials will not be collected or transmitted to the sponsor.

11 Ethics

11.1 Institutional Review Board, Independent Ethics Committee, and Institutional Biosafety Committee

Federal regulations and the ICH guidelines require that approval be obtained from an IRB/IEC/IBC before participation of human subjects in research studies. Before study onset, the protocol, ICF, advertisements to be used for the recruitment of study subjects, and any other written information regarding this study to be provided to the subject or the subject's legal guardian must be approved by an IRB/IEC/IBC. Documentation of all IRB/IEC/IBC approvals and of the IRB/IEC/IBC compliance with ICH harmonised tripartite guideline E6(R2): Good Clinical Practice (GCP) must be maintained by the study site and made available for review by the sponsor or its designee.

All IRB/IEC/IBC approvals should be signed by the IRB/IEC/IBC chair or designee and must identify the IRB/IEC/IBC name and address, the clinical protocol by title or protocol number or both, and the date approval or a favorable opinion was granted.

The investigator is responsible for providing written summaries of the progress and status of the study at intervals not exceeding 1 year or otherwise specified by the IRB/IEC/IBC. The investigator must

promptly supply the sponsor or its designee, the IRB/IEC/IBC, and, where applicable, the institution, with written reports on any changes significantly affecting the conduct of the study or increasing the risk to subjects.

11.2 Ethical Conduct of the Study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki, ICH GCP, and all applicable local laws and regulations.

11.3 Subject Information and Consent

A written ICF in compliance with regulatory authority regulations and 21 CFR §50 shall be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An ICF template may be provided by the sponsor to study sites. If any institution-specific modifications to study-related procedures are proposed or made by the study site, the ICF should be reviewed by the sponsor or its designee or both before IRB/IEC/IBC submission. Once reviewed, the ICF will be submitted by the investigator to his or her IRB/IEC/IBC for review and approval before the start of the study. If the ICF is revised during the course of the study, all active participating subjects must sign the revised form.

Before recruitment and enrollment, each prospective subject or their legal guardian will be given a full explanation of the study and be given the opportunity to read the approved ICF. Once the investigator is assured that the subject or their legal guardian understands the implications of participating in the study, the subject or their legal guardian will be asked to give consent to participate in the study by signing the ICF.

The investigator shall retain the signed original ICF(s) and must provide a copy of the signed original form to the subject or their legal guardian.

12 Investigator's Obligations

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Any change will be reported to the IRB/IEC/IBC but will not require a protocol amendment.

12.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the subject (or the subject's legal guardian), except as necessary for monitoring and auditing by the sponsor, its designee, the FDA, other applicable regulatory agencies, or the IRB/IEC/IBC.

The investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor must be obtained for the disclosure of any said confidential information to other parties.

12.2 Financial Disclosure and Obligations

Investigators are required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR §54. In addition, the investigator must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Neither the sponsor nor PPD is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, neither the sponsor nor PPD is financially responsible for further treatment of the subject's disease.

12.3 Investigator Documentation

Prior to beginning the study, the investigator will be asked to comply with ICH E6(R2) 8.2 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

- IRB/IEC/IBC approvals;
- Original investigator-signed investigator agreement page of the protocol;
- Form FDA 1572 (or equivalent), fully executed, and all updates on a new fully executed Form FDA 1572 (or equivalent);
- Curriculum vitae for the investigator and each subinvestigator;

- Financial disclosure information to allow the sponsor to submit complete and accurate certification or disclosure statements required under 21 CFR §54. In addition, the investigators must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study;
- IRB/IEC/IBC-approved ICF, samples of study site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject or their legal guardian; and
- Laboratory certifications and normal ranges for any local laboratories used by the study site, in accordance with 42 CFR §493.

12.4 Study Conduct

The investigator agrees that the study will be conducted according to the principles of ICH E6(R2). The investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations. Study information from this protocol will be posted on publicly available clinical study registers before enrollment of subjects begins.

Where available, agreed upon by the investigator and allowed by local regulation, an outpatient clinic visit may take place as home health services. **Investigators will be responsible for maintaining effective lines of communication between the investigator and the personnel who manage the subjects at home to guarantee that the investigator is constantly kept informed of subject safety, including metabolic and clinical status.**

12.5 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6(R2) and all applicable guidelines and regulations.

12.6 Adverse Events and Study Report Requirements

By participating in this study, the investigator agrees to submit reports of SAEs according to the time line and method outlined in the protocol. In addition, the investigator agrees to submit annual reports to the study site IRB/IEC/IBC as appropriate.

12.7 Investigator's Final Report

Upon completion of the study, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC/IBC with a summary of the study's outcome and the sponsor and regulatory authority(ies) with any reports required.

12.8 Record Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the study product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

12.9 Publications

After completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, the sponsor will be responsible for these activities and will work with the investigators to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues. The sponsor has final approval authority over all such issues.

Data are the property of the sponsor and cannot be published without prior authorization from the sponsor, but data and publication thereof will not be unduly withheld.

13 Study Management

The administrative structure will include a DMC (Section 9.3).

13.1 Monitoring

13.1.1 Monitoring of the Study

The clinical monitor, as a representative of the sponsor, has the obligation to closely follow the progression of the study, ensuring that it is being conducted in compliance with the protocol, ICH E6(R2), all applicable local laws and regulations, and with current and applicable standard operating

procedures. In doing so, the monitor will visit the investigator and study site at periodic intervals, in addition to maintaining necessary telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and study site personnel.

13.1.2 Inspection of Records

Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB/IEC/IBC review, and regulatory inspections by providing direct access to all study records. In the event of either an audit or inspection, the investigator agrees to allow the sponsor, representatives of the sponsor, or a regulatory agency (eg, FDA or other regulatory agency) access to all study records.

The investigator should promptly notify the clinical research associate (CRA) of any audits or inspections scheduled by any regulatory authorities and promptly forward copies of any reports received to the CRA. The CRA will then inform and forward any reports to the sponsor.

13.2 Management of Protocol Amendments and Deviations

13.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the subject, must be reviewed and approved by the sponsor or its designee. Amendments to the protocol must be submitted in writing to the investigator's IRB/IEC/IBC for approval before subjects can be enrolled into an amended protocol.

13.2.2 Protocol Deviations

A deviation from the protocol is a departure from the written procedures or processes. **A significant deviation occurs when there is nonadherence to the protocol by the subject or investigator that results in a significant, additional risk to the subject or important change to the study design.** Significant deviations can include nonadherence to inclusion or exclusion criteria, nonadherence to safety and efficacy-related assessments, or nonadherence to FDA regulations or ICH GCP guidelines, that could lead to the subject being withdrawn from the study (Section 7.1). Protocol waivers or exemptions are not permitted. Adherence to the study design requirements, including those specified in the Schedules of Events (Table 15-1 and Table 15-2), is essential for study conduct.

The investigator or designee must document and explain in the subject's source documentation any deviation from the approved protocol. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study subjects without prior IRB/IEC/IBC approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB/IEC/IBC for review and approval, to the sponsor for agreement, and to the regulatory authorities, if required.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. Principal investigators will be notified in writing by the monitor of deviations. The IRB/IEC/IBC should be notified of all protocol deviations in a timely manner, as required.

13.3 Study Termination

Although the sponsor has every intention of completing the study, the sponsor reserves the right to discontinue the study at any time for clinical or administrative reasons.

The end of the study is defined as the date on which the last subject completes the last visit (includes follow-up visit).

13.4 Final Report

Whether the study is completed or prematurely terminated, the sponsor will ensure that the clinical study reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The sponsor will also ensure that the clinical study reports in marketing applications meet the standards of the ICH harmonised tripartite guideline E3: Structure and content of clinical study reports.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results.

Upon completion of the clinical study report, the sponsor will provide the investigator with a summary of the cumulative study results. The investigator is encouraged to share the cumulative summary results and will provide each subject with their individual data. The study results will be posted on publicly available clinical study registers, where required.

Declaration of Investigator

I have read and understood all sections of the protocol titled "A Phase 1/2, Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) Serotype 8 (AAV8)-Mediated Gene Transfer of Human Ornithine Transcarbamylase (OTC) in Adults with Late-Onset OTC Deficiency."

I have read and agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the Final Protocol Version 06, dated 25 February 2020, the International Council for Harmonisation harmonised tripartite guideline E6(R2): Good Clinical Practice and all applicable government regulations and inform all who assist me in the conduct of this study of their responsibilities and obligations.

Signature of Principal Investigator	Date

Printed Name of Principal Investigator

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15 Appendices

15.1 Appendix: Schedules of Events

Table 15-1 Schedule of Events – Outpatient, Home Health Services, and Inpatient Clinic Visits

Procedure	Screening	Day 0 (Baseline)	Day 1 (DTX301 Dosing)		Treatment Period: Includes Inpatient Clinic (shaded gray) and Outpatient Clinic/HHS visits. For home visits, refer to Table 15-2.									End of Study/ Early Withdrawal
			IC											
Visit Type	OC	IC	Prior to DTX301 Infusion	DTX301 Dosing/ Postdose	OC/ HHS	OC/ HHS	IC	OC/ HHS	IC	OC/ HHS	OC (all subjects)	IC	OC/ HHS	IC
Week	–	-	-	–	2	4	6	10	12	16	20	24	36	52
Day	–35 to –1	0	1	1	14	28	42	70	84	112	140	168	252	364
Visit Window (Days)	–	–	–		±2	±2	±2	±2	±2	±7	±7	±7	±7	±7
Informed consent	X													
Eligibility criteria	X													
Demographics	X													
OTC medical history	X													
Medical history	X													
Prior medication / therapies / procedures	X													
HBV, HCV, HIV status	X													
Serum pregnancy test (females of childbearing potential only)	X													
Clinic admission		X[a]	X[a]				X[b]		X[b]			X[b]		X[b]
Vital signs (HR, BP, RR)	X	X	X	X[d]	X	X	X	X	X	X	X[e]	X	X	X
Temperature [f]	X		X				X		X		X	X		X
Height	X													

Procedure	Screening	Day 0 (Baseline)	Day 1 (DTX301 Dosing)		Treatment Period: Includes Inpatient Clinic (shaded gray) and Outpatient Clinic/HHS visits. For home visits, refer to Table 15-2.									End of Study/ Early Withdrawal
			IC											
Visit Type	OC	IC	Prior to DTX301 Infusion	DTX301 Dosing/ Postdose	OC/ HHS	OC/ HHS	IC	OC/ HHS	IC	OC/ HHS	OC (all subjects)	IC	OC/ HHS	IC
Week	–	-	-	–	2	4	6	10	12	16	20	24	36	52
Day	–35 to –1	0	1	1	14	28	42	70	84	112	140	168	252	364
Visit Window (Days)	–	–	–		±2	±2	±2	±2	±2	±7	±7	±7	±7	±7
Weight	X	X												X
Urine pregnancy test (females of childbearing potential only)		X			X	X	X	X	X	X	X[e]	X	X	X
Orotic acid spot urine					X	X		X		X	X[e]		X	
24-hour urine orotic acid[g, h]		X					X		X			X		X
Sample for OTC genotyping		X[c]												
Clinical chemistry (including LFTs)[i]	X	X[c]	X	X[j]	X	X	X[c]	X	X[c]	X	X[e]	X[c]	X	X[c]
LFTs (STAT sample at local laboratory)[i]	X	X	X	X[j]	X	X	X	X	X			X		X
Spot ammonia (STAT sample at local laboratory)	X	X[c]	X	X[n]	X	X	X[c]	X	X[c]	X	X[e]	X[c]	X	X[c]
Hematology / coagulation panel	X	X			X		X		X			X		X
Urinalysis	X	X					X		X			X		X

Procedure	Screening	Day 0 (Baseline)	Day 1 (DTX301 Dosing)		Treatment Period: Includes Inpatient Clinic (shaded gray) and Outpatient Clinic/HHS visits. For home visits, refer to Table 15-2.									End of Study/ Early Withdrawal
			IC											
Visit Type	OC	IC	Prior to DTX301 Infusion	DTX301 Dosing/ Postdose	OC/ HHS	OC/ HHS	IC	OC/ HHS	IC	OC/ HHS	OC (all subjects)	IC	OC/ HHS	IC
Week	–	-	-	–	2	4	6	10	12	16	20	24	36	52
Day	–35 to –1	0	1	1	14	28	42	70	84	112	140	168	252	364
Visit Window (Days)	–	–	–		±2	±2	±2	±2	±2	±7	±7	±7	±7	±7
Samples for plasma ammonia (AUC_{0-24})[g,k]		X[l, m]					X[m]		X[m]			X[m]		X[l,m]
Amino acid panel		X[c]					X[c]		X[c]			X[c]		X[c]
AAV8 neutralizing antibody test (cell-based assay)	X	X[c]												X[c]
AAV8 binding antibody IgG assay (ELISA)	X	X[c]												X[c]
Anti-OTC antibody assay (ELISA)		X							X					X
Saliva, urine, and stool for viral shedding [redacted]		X[c]					X[c]	X	X[c]	X	X[e]	X[c]	X	X[c]
Complete PE	X													X
Targeted PE		X					X		X			X		
12-Lead ECG	X	X		X[p]										X
PROMIS questionnaire[q]		X					X		X			X		X
WASI-II vocabulary and matrix reasoning subtests		X[l]												

Procedure	Screening	Day 0 (Baseline)	Day 1 (DTX301 Dosing)		Treatment Period: Includes Inpatient Clinic (shaded gray) and Outpatient Clinic/HHS visits. For home visits, refer to Table 15-2.									End of Study/ Early Withdrawal
			IC											
Visit Type	OC	IC	Prior to DTX301 Infusion	DTX301 Dosing/ Postdose	OC/ HHS	OC/ HHS	IC	OC/ HHS	IC	OC/ HHS	OC (all subjects)	IC	OC/ HHS	IC
Week	–	-	-	–	2	4	6	10	12	16	20	24	36	52
Day	–35 to –1	0	1	1	14	28	42	70	84	112	140	168	252	364
Visit Window (Days)	–	–	–		±2	±2	±2	±2	±2	±7	±7	±7	±7	±7
[redacted]		X[l]												X[l]
[redacted]		X[l]												X[l]
[redacted]		X[l]												X[l]
Oral [1-^{13}C]sodium acetate[r]	X[m]		X[m]				X[m]		X[m]		X[m]	X[m]		X[m]
Samples for ureagenesis[g, s]	X		X				X		X		X	X		X
IWRS	X[t]	X[t]												
DTX301 infusion				X[m, u]										
AE/SAE monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications / therapies / procedures		X	X		X	X	X	X	X	X	X	X	X	X
Review personalized, prescribed diet		X					X		X			X		X
Review of dietary protein intake	X	X	X		X	X	X	X	X	X	X	X	X	X

Procedure	Screening	Day 0 (Baseline)	Day 1 (DTX301 Dosing)		Treatment Period: Includes Inpatient Clinic (shaded gray) and Outpatient Clinic/HHS visits. For home visits, refer to Table 15-2.									End of Study/ Early Withdrawal
			IC											
Visit Type	OC	IC	Prior to DTX301 Infusion	DTX301 Dosing/ Postdose	OC/ HHS	OC/ HHS	IC	OC/ HHS	IC	OC/ HHS	OC (all subjects)	IC	OC/ HHS	IC
Week	–	-	-	–	2	4	6	10	12	16	20	24	36	52
Day	–35 to –1	0	1	1	14	28	42	70	84	112	140	168	252	364
Visit Window (Days)	–	–	–		±2	±2	±2	±2	±2	±7	±7	±7	±7	±7
Review of ammonia scavenger use	X	X	X		X	X	X	X	X	X	X	X	X	X
Consider tapering ammonia scavenger therapy[v]									X			X		

Abbreviations: AAV8, adeno-associated virus serotype 8; AE, adverse event; AUC_{0-24}, area under the curve from time zero to 24 hours; BP, blood pressure; [redacted] ECG, electrocardiogram; ELISA, enzyme-linked immunosorbent assay; HBV, hepatitis B virus; HCV, hepatitis C virus; HHS, home health services; HIV, human immunodeficiency virus; HR, heart rate; IC, inpatient clinic; IgG, immunoglobulin G; IP, investigational product; IWRS, interactive web response system; LFT, liver function test; OC, outpatient clinic; OTC, ornithine transcarbamylase; PE, physical examination; PROMIS, Patient-Reported Outcomes Measurement Information System; [redacted] RR, respiratory rate; SAE, serious adverse event; WASI-II, Wechsler Abbreviated Scale of Intelligence, Second Edition; WAIS-IV, Wechsler Adult Intelligence Scale, Fourth Edition; WMI, Working Memory Index.

Note 1: If **rescreening is within 3 months** of the original screening or **if dosing is expected to occur more than 3 months after completion of original screening** assessments, the following must be repeated: hematology, coagulation and urinalysis, serum pregnancy test (if applicable), clinical chemistry including LFTs, spot plasma ammonia, amino acid panel, AAV8 neutralizing antibody test, weight, and vital signs. Additional tests may be requested on a case-by-case basis, depending on the original reason for screen failure or delay in dosing. **The AAV8 screening neutralizing antibody results must be available and reviewed before IP administration.** If the rescreening occurs more than 3 months after the original screening, all tests must be repeated except the following: OTC genotyping, HBV, HCV, and HIV.

Note 2: At any point between scheduled visits, additional, unscheduled assessment for LFTs, plasma ammonia, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator.

Note 3: At any time after initiation of prophylactic corticosteroid regimen, additional assessments of plasma ammonia levels, amino acid profiles, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator and as clinically indicated.

Note 4: Where available, agreed upon by the investigator and allowed by local regulation, an outpatient clinic visit may take place as home health services. Week 20 visit is a mandatory Outpatient clinic visit for all subjects.

a. Subjects will be discharged 24 hours after the administration of DTX301 on Day 1. Subjects will be inpatient for approximately 48 consecutive hours.
b. Subjects will be discharged after the administration of [1-^{13}C]sodium acetate and after all samples for ureagenesis have been collected; subjects will be inpatient for approximately 28 hours.
c. To be collected/performed before the start of plasma ammonia area under the curve from time zero to 24 hours (AUC_{0-24}) determination.
d. Vital signs to be measured at approximately 5 minutes after the start of DTX301 infusion, and at approximately 0.5 (±5 minutes), 1 (±5 minutes), 2, 4, 6, 8 (±15 minutes), and 22 hours (±1 hour) after the start of DTX301 infusion.
e. To be collected prior to the [1-^{13}C]sodium acetate administration.
f. Temperature will be measured prior to administration of [1-^{13}C] sodium acetate. If the temperature is > 101°F or > 38°C, the procedure should not be initiated.
g. Collection of samples for AUC_{0-24} of plasma ammonia and urine orotic acid determination is to be performed prior to administration of [1-^{13}C]sodium acetate and sample collection for determination of the rate of ureagenesis.
h. Urine samples to be collected at time 0 and at approximately 6, 12, 18, and 24 hours (relative to the start of plasma ammonia determination). Urinary orotic acid concentration will be standardized to urine creatinine concentration.
i. Through Week 12, one sample for LFTs is collected as part of clinical chemistry and sent to the central laboratory for analysis. A second sample for LFTs only is collected and sent to the local laboratory (STAT sample).
j. Samples for clinical chemistry to be collected at approximately 0.5, 4, 8, and 22 hours after the start of DTX301 infusion.
k. Samples to be collected at time 0 and at approximately 2, 4, 8, 12, 16, 20, and 24 hours (relative to start of plasma ammonia determination). Two samples for plasma ammonia to be collected at each time point; 1 sample will be analyzed at the on-site local laboratory and the second sample will be processed and sent to the central laboratory.
l. The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable in order to perform the neuropsychological tests. The 0-hour plasma ammonia sample (for AUC_{0-24} of plasma ammonia) local laboratory result (STAT sample) may be used to confirm plasma ammonia levels. The results must be back prior to starting the neuropsychological tests.
m. The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable in order to receive [1-^{13}C]sodium acetate and DTX301 (Day 1 only). If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. If the subject is deemed clinically unstable, [1-^{13}C]sodium acetate and DTX301 (Day 1 only) will be held until the subject is determined to be clinically stable. Rescreening procedures may apply (Screening and Day 1 only). On Day 1 and at Weeks 6, 12, 24, and 52, the 24-hour (T 24) plasma ammonia from the local (STAT sample) may be used. The result must be back prior to dosing with [1-^{13}C]sodium acetate and DTX301 (Day 1 only). On Day 1, the same T 24 local laboratory (STAT sample) can serve as the DTX301 predose plasma ammonia result, if drawn within 12 hours or less of dosing with DTX301. If not, a new plasma ammonia (STAT sample) is to be collected prior to dosing with DTX301.
n. A single sample for plasma ammonia (STAT sample at local laboratory) to be collected and reviewed prior to discharging the subject after DTX301 infusion.

o. Viral shedding determination in saliva, stool, and urine to be performed at Weeks 6, 10, and 12 and on Days 46, 58, 62, and 78 (Table 15-2). Samples will be collected until negative on 3 consecutive occasions. Subjects will be provided an appropriate container to collect a stool sample at home.

p. 12-Lead ECG to be performed at approximately 1 hour after the start of DTX301 infusion.

q. PROMIS questionnaire to be completed prior to neuropsychological tests on Day 0 and at Week 52.

r. At inpatient clinic visits, [1-^{13}C]sodium acetate is to be administered orally on the second day of inpatient admission, after all samples for AUC_{0-24} plasma ammonia and orotic acid determination (over 24 hours) have been collected. Prior to administering [1-^{13}C]sodium acetate, the site must confirm the subject's plasma ammonia level. The 24-hour (T 24) plasma ammonia from the local laboratory (STAT sample) may be used. The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable. If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. If the subject is deemed clinically unstable, [1-^{13}C]sodium acetate will be held until the subject is determined to be clinically stable. On Day 1, the same local laboratory (STAT sample) can serve as the DTX301 predose plasma ammonia used for this assessment should be drawn within 12 hours or less of dosing with DTX301. If not, a new plasma ammonia (STAT sample) is to be collected prior to dosing with DTX301. **Subjects will fast for at least 6 hours, including liquids containing protein, sugar or carbonate, prior to administration of [1-^{13}C]sodium acetate. After dosing, subjects will continue to fast for at least 4 hours. Water is allowed *ad libitum*.**

s. Samples to be collected before dosing with [1-^{13}C]sodium acetate (time 0) and at approximately 0.5, 1, 1.5, 2, 3, and 4 hours after dosing with [1-^{13}C]sodium acetate. Subjects will fast for at least 6 hours, including liquids containing protein, sugar or carbonate, prior to administration of [1-^{13}C]sodium acetate. After dosing, subjects will continue to fast for at least 4 hours. Water is allowed ad libitum. During Screening, assessment of rate of ureagenesis may be repeated if discrepant with subject's clinical status and severity.

t. Once eligibility is confirmed, the study site should register the visit with IWRS. Study personnel must schedule the dosing visit in IWRS ideally 7 days but no less than 3 days prior to the actual visit date at the study site in order to allow for adequate shipment time and delivery of DTX301 to the study site.

u. The start of DTX301 infusion should be after all samples for the determination of the rate of ureagenesis have been collected. Prior to the start of DTX301 infusion, the study site must confirm that the subject's plasma ammonia level on Day 1 (predose) is < 100 µmol/L for patients who historically maintain normal ammonia levels, and the subject is clinically stable; OR the subject's plasma ammonia level on Day 1 (predose) is < 200 µmol/L, for patients who historically are not able to fully control ammonia levels with baseline management, and the subject is clinically stable. If the Day 1 (predose) ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. NOTE: If the subject is deemed clinically unstable, dosing will be held, and the subject can be rescreened once the subject is determined to be clinically stable. The same 24-h time point (T 24) from local laboratory (STAT sample) AUC_{0-24} of plasma ammonia can serve as the DTX301 predose plasma ammonia result, if drawn within 12 hours or less of dosing with DTX301. If not, a new plasma ammonia (STAT sample) is to be collected prior to dosing with DTX301.

v. Adjustments to ammonia scavenger therapy may be considered following the Week 12 and Week 24 visits. The subject must be clinically stable and under good metabolic control before changes can be initiated or progressed. The risks of making adjustments to baseline treatment on their own, without express guidance from the site, will be reinforced with the subject at site visits. Modification of ammonia scavenger therapy cannot occur at the same time as changes in protein-restricted diet. Changes to baseline treatment cannot occur while the subject is treated with corticosteroids or within a 2-week period of completing a corticosteroid taper. Modification of baseline treatment will be individualized based on review of the totality of longitudinal clinical and laboratory data for each subject, including ammonia levels, plasma ammonia AUC_{0-24}, subject clinical stability/asymptomatic status, neurocognitive status, and subject-reported outcomes. **Rate of ureagenesis cannot be used for decision-making in modification of ammonia scavenger therapy or protein-restricted diet and results will not be made available to the investigative sites until the end of the study.**

Table 15-2 Schedule of Events – Clinic or Home Visits During the Initial 12-Week Treatment Period

		Treatment Period												
	Week	1	2	3	4	5	6	7	8		9		10	11	12
	Day	5	9	18	22	32	36	46	50	54	58	62	66	74	78
Procedure	Visit Window (Days)	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1
Clinical chemistry (including LFTs)[a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
LFTs (local laboratory, STAT sample)[a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Spot ammonia (local laboratory STAT sample)[b]		X	X		X		X		X		X		X	X	
Saliva, urine, and stool for viral shedding [redacted]								X			X	X			X

Abbreviations: LFT, liver function test; [redacted].

Note: At any time after initiation of prophylactic corticosteroid regimen, additional assessments of plasma ammonia levels, amino acid profiles, or any other biomarker to assess subject safety and clinical status may be performed, at the discretion of the investigator and as clinically indicated.

a. Through Week 12, one sample for LTFs is collected as part of clinical chemistry and sent to the central laboratory for analysis. A second sample for LFTs only is collected and sent to the local laboratory (STAT sample).

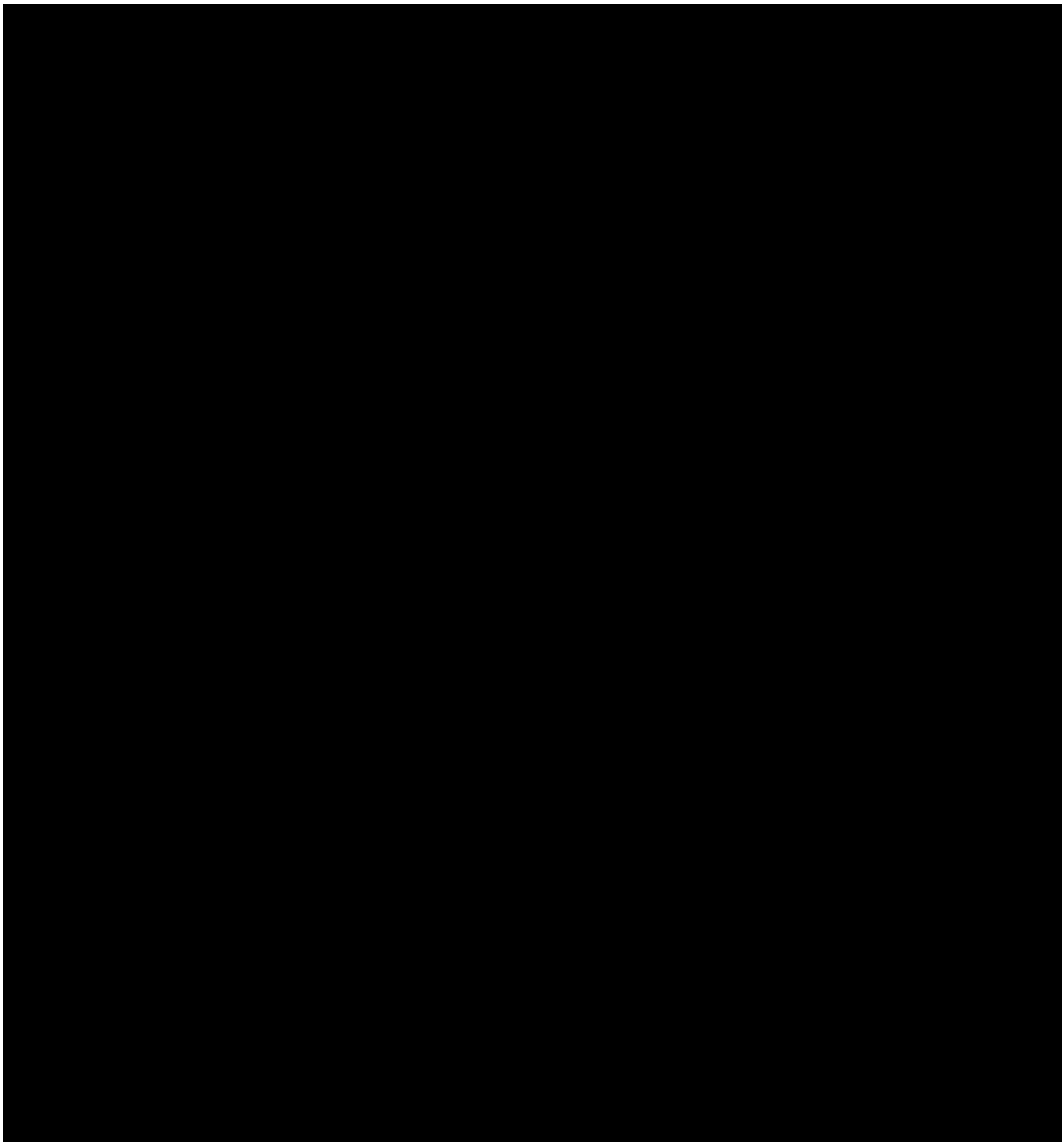
b. Through Week 12, one sample for spot ammonia is collected approximately once a week and sent to the local laboratory (STAT sample).

c. Samples for viral shedding are to be collected at Weeks 6, 10, and 12 (see Table 15-1) and on Days 46, 58, 62, and 78 until negative on at least 3 consecutive occasions for each matrix.

15.2 Quality of Life Questionnaire

15.2.1 PROMIS Questionnaire

[redacted]



English
March 6, 2014

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15.3 Protocol Amendment History and Summary of Changes

15.3.1 Amendment 1 (dated 18 August 2016)

The primary objective of Amendment 1 of the protocol, 301OTC01, is to address feedback received from the US Food and Drug Administration, the Medicines and Healthcare Products Regulatory Agency, and the National Institutes of Health Recombinant DNA Advisory Committee.

An overview of changes includes the following:

- Section 1.3.1 (Design Rationale) has been updated to include rationale for defining hyperammonemia as ≥100 μmol/L.
- The dosing interval between subjects within a cohort (14 days [Cohorts 1 and 2] and 7 days [subsequent cohorts]) has been revised. Additionally, Section 1.3.1 (Design Rationale) has been updated to include rationale for this change.
- Section 1.3.2 (Dosing Rationale) has been revised to provide rationale for the new starting dose of 2.0×10^{12} genome copies (GC)/kg.
- The rate of ureagenesis will now be the main efficacy parameter to identify the optimal biological dose (OBD) of DTX301. The AUC_{0-24} for serum ammonia will still be evaluated to help determine the efficacy of DTX301 (Section 2).
- The candidate doses to determine the OBD of DTX301 have been revised as follows (Section 3.1):
  - Dose 1: 2.0×10^{12} GC/kg (new starting dose)
  - Dose 2: 6.0×10^{12} GC/kg
  - Dose 3: 1.0×10^{13} GC/kg
- The number of inpatient visits has been reduced from 8 to 5 to alleviate undue burden to subjects (Section 3.2.3).
- The timing for tapering or discontinuing ammonia scavenger therapy has been revised. Investigators will now consider an adjustment to ammonia scavenger therapy following the visits at Week 12 and Week 24, after the most recent monitoring results are available (Section 3.2.4).
- The study stopping criteria have been revised based on feedback received from regulatory authorities (Section 3.2.5).

- Inclusion criterion #2 has been updated to include specific examples of enzymatic, biochemical, or molecular testing that may be performed to confirm ornithine transcarbamylase (OTC) deficiency (Section 4.1).
- Inclusion criterion #5 has been added to ensure that the subject's OTC deficiency is stable prior to receiving DTX301 (Section 4.1).
- Inclusion criterion #10 was removed and incorporated into exclusion criterion #13 to have one cohesive criterion for excluding subjects that are pregnant (Section 4.2).
- Due to reduction of inpatient visits, the rate of ureagenesis will now be measured at Screening, on Day 1, and at Weeks 6, 12, 20, 24, and 52 (Section 8.1.1). Additionally, if clinically indicated, the rate of ureagenesis can be measured at any outpatient visit (scheduled or unscheduled)
- The decision criteria for determining whether to start steroids for suspected vector-induced hepatitis have been revised (Section 8.2.4.1.2).
- The overall number of neuropsychological evaluations that will be performed has been reduced based on feedback from regulatory authorities (Section 8.5).
- Weeks 18 and 22 have been added as a clinic or home visit to collect samples for clinical chemistry.
- Specifying that subjects will receive a prescribed diet during each inpatient stay and that an adjustment may be made to the subject's prescribed diet has been removed from the protocol.
- Minor editorial revisions.

15.3.2 Amendment 2 (dated 20 December 2016)

The primary objective of Amendment 2 of the protocol, 301OTC01, is to address feedback received from the US Food and Drug Administration.

An overview of changes includes the following:

- Clarified that ammonia will be analyzed in plasma, not serum (global protocol revision).
- Clarified that plasma ammonia levels must be <100 µmol/L prior to both administration of the neuropsychological tests (Section 8.5) and [1-^{13}C]acetate administration (Section 6.3.1).
- Revised the language regarding tapering of ammonia scavenger therapy following feedback from the FDA (Section 3.2.4).

- Added language regarding retesting of liver function tests in the event that possible vector-induced hepatitis is suspected following feedback from the FDA (Section 8.2.4.1.2).
- Added collection of samples to test for antibodies against ornithine transcarbamylase.
- The schedule of events has been revised, as needed (Table 15-1).
- Minor editorial revisions.

15.3.3 Amendment 3 (dated 07 February 2017)

The primary objective of Amendment 3 of the protocol, 301OTC01, is to address feedback received from the United Kingdom (UK) Medicines and Healthcare Products Agency (MHRA) and the Gene Therapy Advisory Committee (GTAC).

An overview of changes from Amendment 2 (20 December 2016) to Amendment 3 (07 February 2017) includes the following:

- Added UK MHRA-requested language around definition of abstinence as a form of birth control (Section 4.1).
- Per UK MHRA request, added that following data monitoring committee (DMC) review when a stopping rule is met, if the decision is to restart enrollment, a substantial amendment will need to be approved by the regulatory authority (Sections 3.2.5 and 9.3).
- Per GTAC request, provided further justification and clarification that the neuropsychological battery of testing is an important assessment for OTC deficiency patients and beyond this 52-week study, it will be performed every 2 years during the 4-year extension study for each study subject (Section 8.5).
- Per GTAC request, added language allowing study subjects to request rest time during administration of the neuropsychological battery (Section 8.5).
- Per study sites' request, clarified that the predose plasma ammonia used to determine DTX301 dosing must be drawn within 12 hours or less of dosing (Section 3.2.2.2).
- Per study sites' request, clarified wording to reflect that flexibility in performing the ureagenesis test first is allowed so long as it does not overlap temporally with the plasma ammonia AUC_{0-24} and urinary orotic acid levels over the 24-hour collection period (Section 3.2.2.1).

- Per Dimension Therapeutics (DMTX), deleted inclusion criterion #7 since there is no requirement to taper ammonia scavenger medications (Section 4.2).

- Per DMTX, corrected the exclusion criterion (#9) for the anti-AAV8 neutralizing antibody titer to ≥ 1:5 from > 1:5 (typographical error) (Section 4.2).

- Per DMTX, clarified rescreening of patients (Section 3.2.1).

- Per DMTX, changed the start of the DMC assessment for dose escalation from all subjects in a dose cohort having completed Week 6, to all subjects in a dose cohort having completed Week 12 (Section 1.3.1).

- Per DMTX, changed the alanine aminotransferase (ALT) threshold for considering starting steroid treatment from 2.5 × upper limit of normal (ULN), to greater than the ULN (Section 8.2.4.1.2). Therefore, exclusion criterion #4 adjusted to ALT or aspartate aminotransferase > ULN from > 2.0 × ULN (Section 4.2).

- Per DMTX, increased enzyme-linked immunospot assessments to approximately weekly during already scheduled visits through Week 12 for more frequent monitoring of immune response (Section 8.2.5.2 and Table 15–1).

- Per DMTX, added liver function tests to be performed at local laboratory (STAT sample) during the scheduled collections approximately every 4 days up through Week 12 in the interest of closer monitoring and faster turnaround of liver function testing results (Section 8.2.4.1.1, Table 8–1, and Table 15–1).

15.3.4 Amendment 4 (dated 11 June 2019)

The primary objective of Amendment 4 of the protocol, 301OTC01, is to provide details on the inclusion of the additional subjects as expansion of Cohort 3, already foreseen in the original protocol, and to add Cohort 4 (Dosing Process Optimization). Subjects in Cohort 4 will receive DTX301 at the optimal biological dose and will receive a prophylactic corticosteroid regimen, intended to achieve prophylaxis of vector-induced hepatitis.

An overview of changes from Amendment 3 (07 February 2017) to Amendment 4 (11 June 2019) includes the following:

- The sponsor name was updated globally from Dimension Therapeutics, Inc. to Ultragenyx Pharmaceutical Inc.

- The sponsor signatory was updated globally to [redacted] MD, PhD.

- The medical monitor was updated to [redacted] MD.

- An ongoing AAV8 gene transfer clinical study in Crigler-Najjar syndrome, which uses a prophylactic steroid regimen, was added as a reference and cited as applicable (Sections 1.2, 8.2.4.1.2).

- The generation of fewer empty particles via a DTX301 manufacturing process, which was presented as a difference between this clinical study and previous gene transfer studies, was removed for accuracy (Section 1.2).

- The changes detailed in Addendum A to Amendment 3 (dated 02 March 2017) were incorporated. The purpose of Addendum A was to align Figure 3–1 Study Design with the main text of the study protocol (Section 3.2).

- The changes detailed in the country-specific Addendum B to Amendment 3 (dated 21 April 2017) for sites in the United Kingdom were incorporated. The purpose of the UK-specific Addendum B was to add the requirement that no subjects from UK sites can be dosed until at least 42 days after the first subject in the study (ie, first subject in Cohort 1) has been dosed. This change was made to address feedback received from the Gene Therapy Advisory Committee (GTAC) (Sections 1.3.1, 3.1, Figure 3–1).

- The process for enrollment of additional subjects to Cohort 3 (Cohort Expansion) and an additional cohort (Cohort 4 [Dosing Process Optimization] was added (Sections 1.3.1, 3.1, 3.2, 5.2, 10.5.1).

- A reference to ongoing safety results from this study (Study 301OTC01) was added (Sections 1.3.1, 14).

- An ongoing AAV gene transfer clinical study in hemophilia B, which is dosing patients at a minimum of 1 day apart, was added as a reference and cited as applicable (Section 1.3.1).

- The details of the prophylactic corticosteroid regimen to be implemented for Cohort 4 (Dosing Process Optimization) were added (Sections 1.3.1, 3.1, 8.2.4.1.1, 8.2.4.1.2).

- The screening period was extended and will begin at Day –35 (Sections 3.1, 3.2.1, 15.1, Table 15-1, Figure 3–1).

- The decision to adjust or taper ammonia scavenger medications was updated to be made based on the investigator's review of the totality of clinical and laboratory data (Sections 3.2.4, 15.1, Table 15-1 [footnote v]).

- Instructions for dose preparation and administration of [1-^{13}C] were updated according to the manufacturer and referenced to the ureagenesis manual (Sections 6.2.5, 6.3.1).

- The duration of fasting before and after administration of [1-^{13}C] sodium acetate was added (Sections 8.1.1, 15.1, Table 15-1 [footnotes r and s]).

- The timing of DMC meetings was updated to account for additional subjects and 1 additional cohort (Sections 9.3, 10.5.1).

- Text was removed that stated the statistical analysis plan will be finalized prior to the start of the study (Section 10).

- The definition of the baseline rate of ureagenesis was updated (Section 10.5.2.1).

- The presentation of other laboratory results and neuropsychological tests was updated to reflect the planned data presentation (Sections 10.5.3.6, 10.5.4)

- Flexibility was added to the timing of the interim analysis. The statement that interim analyses would not bias the conduct of the study was removed (Section 10.5.7).

15.3.5 Amendment 5 (dated 25 February 2020)

The primary objective of Amendment 5 of the protocol, 301OTC01, is to address written requests from the US Food and Drug Administration to provide more comprehensive guidance on monitoring of ammonia levels throughout the study, guidance to investigators on monitoring hyperammonemic crises, and more explicit guidance to investigators regarding tapering and discontinuation of ammonia scavenger medications and protein-restricted diet.

An overview of changes from Amendment 4 (11 June 2019) to Amendment 5 (25 February 2020) includes the following:

- The medical monitor was updated to [redacted] MD to accurately reflect study personnel (Title Page).

- The protocol amendment history and summary of changes were moved from the beginning of the protocol (ie, directly after the protocol approval page) to Appendix 15.3. This change was administrative in nature and does not change the content of the protocol (Section 15.3).

- The age cutoff for presentation of disease signs and symptoms, which is used to delineate neonatal-onset from late-onset ornithine transcarbamylase (OTC) deficiency, was updated from ≤ 1 month of age (neonatal onset) and ≥ 1 month of age (late onset) to ≤ 30 days of age (neonatal onset) and > 30 days of age (late onset). This change was made to align with the DTX301 clinical development documents and cited literature (Sections 1, 4.1).

- The term hyperammonemic crisis (HAC) was introduced and defined explicitly to align with the Urea Cycle Disorders Consortium definition. This addition will ensure standardized monitoring of HACs throughout the remainder of this study (Sections 1, 1.3.1. 2, 3.2.6, 10.4).

- The list of AAV8 clinical studies that are referenced in the introduction were updated to align with the Investigator's Brochure and the most recent information (Section 1.3.1)

- The timing of dosing for subjects in the United Kingdom in Cohort 1 was removed because Cohort 1 enrollment is now completed (Section 1.3.1, Figure 3-1).

- The observed timing of elevations in liver enzymes following DTX301 administration was updated from "5 to 7 weeks" to "8 days to 7 weeks" after vector administration to reflect ongoing data from this Phase 1/2 study (301OTC01; Section 1.3.1).

- An additional clinical trial (ClinicalTrials.gov identifier: NCT02716246) was referenced as justification for the prophylactic steroid regimen to be introduced in Cohort 4 (Dosing Process Optimization; Sections 1.3.1, 8.2.4.1.2).

- The definition of baseline for the rate of ureagenesis was removed globally with the intent that baseline definitions will be presented in the statistical analysis plan (Sections 2, 10.5.2.1).

- The text, "in the setting of tapering or discontinuing ammonia scavenger medications," was removed from exploratory endpoints 2 and 3 (ie, urinary orotic acid excretion and glutamine and glutamate). The change was made because the intent is to assess these endpoints in all subjects, regardless of baseline treatment status (Section 2).

- The specified timing, "weekly," was removed from the exploratory endpoint that evaluates use of ammonia scavengers. This change was made to reflect the manner by which the data are being captured and reported and to reflect subject management, which requires daily intake of scavengers at a prescribed dose (Sections 2, 8.7.2, 10.4).

- The exploratory endpoints and corresponding assessments determined by the enzyme-linked immunospot [ELISPOT] assay and related to cell-mediated immune response to AAV8 and OTC were removed globally. These assays are exploratory in nature and to date have not revealed informative results, and require notable blood volume to be collected from subjects (Sections 2, 8.2, 8.2.4.1.1, 10.4, 10.5.3.6, Table 15-1).

- The sample size was updated from "approximately 6 to 18 subjects" to "up to 18 subjects" to reflect the current enrollment status of 9 subjects and plans to continue enrollment (Section 3.2).

- It was added that "where available, agreed upon by the investigator and allowed by local regulation, an outpatient clinic visit may take place as home health services." The Week 20 visit remains a mandatory outpatient clinic visit. This change was made to allow increased flexibility for protocol-specified abbreviated visits that may be performed as home health visits and have the potential to reduce subject burden in certain situations (Sections 3.2, 3.2.3, 3.2.3.2, 12.4, Figure 3-1, Table 15-1).

- More comprehensive guidance and testing specifications were added to the rescreening procedure to ensure consistent testing and evaluations prior to enrollment and dosing (Section 3.2.1, Table 15-1).

- The rules for administering [1-^{13}C]sodium acetate and neuropsychological tests were updated as follows: The subject's plasma ammonia level should be < 100 µmol/L or within the range of historical ammonia levels obtained when the subject was clinically stable in order to receive [1-^{13}C]sodium acetate. If the ammonia level is inconsistent with a subject's clinical status, the ammonia level may be repeated to ensure accurate results. This change was made to better reflect patient status with long-standing metabolic and clinical management (Sections 3.2.1, 6.3.1, 8.3.2, 8.5, Table 8-1, Table 15-1).

- Inclusion criterion #5 and globally affected text presenting the ammonia criteria prior to the start of DTX301 administration were updated as follows: Subject's plasma ammonia level on Day 1 (predose) is < 100 µmol/L, for patients who historically maintain normal ammonia levels, and the subject is clinically stable; OR the subject's plasma ammonia level on Day 1 (predose) is

< 200 µmol/L, for patients who historically are not able to fully control ammonia levels with baseline management, and the subject is clinically stable. If the Day 1 (predose) ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. This change was made to better reflect patient status with long-standing metabolic and clinical management (Sections 3.2.2.2, 4.1, 5.1, Table 15-1).

- Exclusion criterion #1 was updated as follows: At Screening or Baseline (Day 0), plasma ammonia level ≥ 100 µmol/L for patients who historically maintain normal ammonia levels; OR plasma ammonia level ≥ 200 µmol/L for patients who historically are not able to fully control ammonia levels with baseline management; OR signs and symptoms of hyperammonemia, with documented elevated ammonia level, during the 4-week period preceding Day 0. If the ammonia level is inconsistent with the subject's clinical status, the ammonia level may be repeated to ensure accurate results. This change was made to better reflect patient status with long-standing metabolic and clinical management (Section 4.2).

- It was added that the assessment of rate of ureagenesis may be repeated during Screening if discrepant with subject's clinical status and severity. The rate of ureagenesis is a direct *in vivo* assessment of the efficiency of the urea cycle and therefore should be compatible with patient severity given the understanding that neither ammonia scavenger therapy nor protein-restricted diet directly alters the cycle (Sections 3.2.1, 8.1.1, 8.3.1, 10.5.2.1, Table 15-1).

- The details of obtaining spot plasma ammonia prior to dosing were clarified, and the requirement for a plasma ammonia (STAT sample) within 12 hours of DTX301 dosing was added explicitly (Section 3.2.2.2, Table 15-1).

- It was added that at any point between scheduled visits (and after initiation of a prophylactic steroid regimen) additional testing of liver function tests, plasma ammonia, or other biomarkers to assess subject safety and clinical status may be performed, at the discretion of the investigator. This text was added to make it explicit that investigators have the flexibility to perform additional testing and that subject safety is being monitored closely throughout the study (Sections 3.2.3, 3.2.4, 8.2.4, 8.2.4.1, 8.2.4.1.2, Table 15-1).

- Spot ammonia testing was made explicit and added to additional visits to ensure ammonia is being monitored closely throughout the clinical study (Sections 3.2.3.1, 8.2, Table 15-1, Table 15-2).

- Text was added to explicitly state that subjects will be re-educated on the risks of making adjustments to baseline treatment on their own, without the clear guidance of the study site investigator (Sections 3.2.4, 8.7.2, Table 15-1).

- Additional rules for changes to baseline treatment (ie, ammonia scavenger medication and protein-restricted diet) were added to ensure standardized implementation. The additional rules specify that changes to baseline treatment cannot occurring during or within 2 weeks of corticosteroid treatment. Changes to ammonia scavenger medication and protein-restricted diet must not occur at the same time (Sections 3.2.4, 3.2.5, 8.2.4.1.2, 8.7.2, Table 15-1).

- Explicit text was added that rate of ureagenesis cannot be used for decision-making in modification of ammonia scavenger therapy or protein-restricted diet and results will not be made available to the investigative sites until the end of the study (Sections 3.2.4, Table 15-1).

- Specific guidance was added for considering reinstitution of ammonia scavenger therapy and for implementing a second round of modification of baseline treatment. This guidance was added to ensure standardized modifications and assurance of subject safety (Sections 3.2.4).

- Additional guidance was added for modification of protein-restricted diet. This guidance was added to ensure standardized modifications and assurance of subject safety (Section 3.2.5).

- Inclusion criterion #6 was updated to specify that a stable dose of ammonia scavenger therapy must be "ongoing daily." This text was added to ensure the intent of this criterion was explicitly stated (Section 4.1).

- The number of medical personnel (in addition to the study site pharmacist) who must check the dosing calculations for [1-^{13}C]sodium acetate was updated from "a minimum of 2 medical personnel" to "a member of medical personnel" (Section 6.3.1).

- Treatment compliance was updated to include adherence to ammonia scavenger medication regimens and protein-restricted diet (Section 6.3.3).

- The assessment of vector genome determination was removed globally. The results to date for this parameter, including the highest dose planned to be administered for the DTX301 program, have consistently demonstrated sequential reduction of blood viral load without rebound in values; therefore, based on medical consideration of results to date and to reduce subject burden, additional testing and collection of subject blood is not warranted (Sections 8.2, 10.5.3.6, Table 15-1).

- Electrocardiogram testing was removed from predose on Day 1 to reduce subject burden and based on medical evaluation of study results to date (Section 8.2.3).

- It was added that prior to initiating the prophylactic steroid regimen (Cohort 4), the subject must be assessed as clinically and metabolically stable, and intercurrent illnesses (eg, viral infection) or concomitant medications known to affect transaminases, be excluded (Section 8.2.4.1.2).

- Additional guidance was added to specify when ad hoc 24-hour plasma ammonia assessments should be considered to ensure subject safety is paramount (Section 8.3.2).

- It was explicitly stated that hospitalization due to hyperammonemic crisis will be considered a serious adverse event (Section 9.1.1.2).

- It was added that adverse events will be assessed in terms of the relationship to corticosteroid regimen, in addition to the previously included assessments of relationship (eg, study product, [1-^{13}C] sodium acetate, OTC deficiency, or hyperammonemia). This update was made per a request from the Data Monitoring Committee (Sections 9.1.2.1, 9.1.4).

- The version of the NCI CTCAE to be used was updated from Version 4.03 to the most current version (Sections 9.1.3, 14).

- An Data Monitoring Committee meeting was introduced after completion of Week 12 for the first 3 subjects dosed in Cohort 4. This meeting was adjusted per a request from the Data Monitoring Committee (Section 9.3).

- The analysis of rate of ureagenesis was updated to include the relative percentage to normal healthy adults (Section 10.5.2.1).

- The analysis of plasma ammonia was updated to include time-normalized plasma ammonia (Section 10.5.2.2).

- Viral shedding, AAV8 neutralizing antibody, and AAV8 binding antibody IgG assay (ELISA) assessments were reduced based on medical consideration of results to date and to reduce subject burden (Table 15-1, Table 15-2).