Statistical Analysis Plan

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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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Statistical Analysis Plan Amendment

Summary of Changes

The original 301OTC01 Statistical Analysis Plan (Version 1.0, dated 11 May 2017) was amended 3 times. Major changes in each SAP version are listed in Table 1.

Table 1: Summary of SAP Versions (Original SAP Version 1.0, Dated 11 May 2017)

Version (Version Date)	Summary of Changes
Version 2.0 (10 November 2017)	• Section 3.1, Overall Study Design and Plan: Clarified that DMC review of a cohort will occur following the collection of a minimum of 12 weeks (84 days) of safety data (with at least 6 weeks of supplementary efficacy data). • Section 3.2, Implementation of the N-CRM Model: Revised section to define the proposed N-CRM model for the study and to provide the details necessary for its implementation. • Section 4.1, Baseline and Change from Baseline: Added a rule for deriving the baseline value for absolute ^{13}C-urea $AUC_{0\text{-}240min}$ percent of normal. • Section 4.15, Analysis Sets: Added the MTD Determining Set. • Section 8.1, Rate of Ureagenesis: Updated the method for estimating the rate of ureagenesis for individual subjects. For summary statistics and the longitudinal plot of change from Baseline, removed the time points 0.5, 1, 1.5, 2, and 3 hours relative to [1-^{13}C]sodium acetate administration. • Section 8.3.1, Supplementary Ureagenesis Parameters: Removed absolute ^{13}C-urea $AUC_{0\text{-}240min}$ from parameters to be derived. Removed units conversion factor for absolute ^{13}C-urea $AUC_{0\text{-}240min}$. For summary statistics, added absolute ^{13}C-urea $AUC_{0\text{-}240min}$ percent of normal. • Section 9.1, Adverse Events: Removed summaries by System Organ Class and Preferred Term for the following categories: TEAEs leading to study discontinuation, serious TEAEs by relationship, and TEAEs with Grade ≥ 3.
Version 3.0 (08 July 2021)	• Updated Sponsor name from Dimension Therapeutics, Inc. to Ultragenyx Pharmaceutical Inc. • Section 2, Objectives and Endpoints: Updated to align with Protocol Amendment 5. • Section 3.1, Overall Study Design and Plan: Updated to align with Protocol Amendment 5. • Section 3.2, Implementation of the N-CRM Model: Added the definition of DLT. Added that Cohort 3 expansion and Cohort 4 will not be included in the dose-finding assessment. • Section 3.3, Treatments: Added Cohort 4 (1.0×10^{13} GC/kg plus prophylactic corticosteroids). Added that efficacy data will be modeled by dose level (ie, Cohorts 3 and 4 will be combined) and that other data will be summarized and reported by cohort. • Section 4.1.1, Ureagenesis Baseline: Updated the rules for deriving baseline values for ureagenesis parameters. Clarified that, when deriving baseline values for non-AUC supplementary ureagenesis parameters, Day 1 results will not be taken into

	consideration if excluded per the rule for deriving the baseline value for the rate of ureagenesis. Added that derived and reported ureagenesis parameters will be summarized. • Section 4.1.2, Other Parameters Baseline: Added spot ammonia and urinary orotic acid $AUC_{0\text{-}24hr}$. • Section 4.1.3, Change from Baseline: Updated the formula for calculating percent change from Baseline. • Section 4.9, Sample Size: Updated to align with Protocol Amendment 5. • Section 4.14, Unscheduled Visits: Added summaries of worst postbaseline value for hematology, chemistry, urinalysis, and amino acid parameters. Added that unscheduled visits will be included in figures for individual subjects. • Section 5.1, COVID-19: Added that no changes to planned analyses due to the impact of COVID-19 were made. Added pandemic start and end dates for participating countries. • Section 5.2, Disposition: Added that all disposition-related information for enrolled subjects will be listed. • Section 6.1, Demographics: Added that Screening and Baseline weight will be summarized. Added that the number of subjects by age category will be displayed. Added that all demographic data and baseline characteristics for subjects in the Safety Set will be listed. • Section 6.2, Medical History: Added that medical history data will be sorted in alphabetical order by System Organ Class and that Preferred Terms will be sorted in descending order by frequency within each System Organ Class. Added that medical history data will be listed. • Section 6.3, OTC Deficiency Medical History: Time since OTC deficiency became stable and time since stable dose of ammonia scavenger therapy will be reported in weeks instead of years. OTC deficiency medical history data for subjects in the Safety Set will be listed. • Section 7.1 Prior and Concomitant Medications: Prior and concomitant medications for subjects in the Safety Set will be listed. • Section 7.1.1, Prior Medications and Section 7.1.2, Concomitant Medications: Medications will be sorted in descending order by ATC level 4 term based on counts in the overall column. • Section 7.3, [1-^{13}C]Sodium Acetate: Added section. Details related to [1-^{13}C]sodium acetate administration for subjects in the Safety Set will be listed. • Section 8.1, Rate of Ureagenesis: Specified that MMRM outputs will include estimates for between-DTX301 dose (overall and by visit) comparisons. Removed summary statistics for rate of ureagenesis and longitudinal plot of change from Baseline to 4 hours postdose in rate of ureagenesis. Removed Yudkoff 2017 reference. • Section 8.2, Plasma Ammonia Area Under the Curve from Time 0 to 24 Hours: Summary statistics and listings will include time-normalized plasma ammonia $AUC_{0\text{-}24hr}$, which will be calculated by dividing AUC by the actual number of minutes between the 0- and 24-hour time points and multiplying by 60. Added the geometric mean, CV%, and geometric CV% for plasma ammonia $AUC_{0\text{-}24hr}$ and time-normalized plasma ammonia $AUC_{0\text{-}24hr}$ by visit. Added the mean and standard deviation for the logarithmic transformation of plasma ammonia $AUC_{0\text{-}24hr}$ and time-normalized plasma ammonia $AUC_{0\text{-}24hr}$. Added a linear MMRM to provide a comparison vs Baseline of the logarithmic transformation of plasma ammonia $AUC_{0\text{-}24hr}$ for each DTX301 dose. Updated the list of figures to be generated.

	• Section 8.3.1, Supplementary Ureagenesis Parameters: Removed ^{13}C-bicarbonate as a parameter to be derived. Added a linear MMRM to evaluate the association between change from Baseline in absolute ^{13}C-urea $AUC_{0\text{-}240min}$ and DTX301 dose. Added a linear MMRM to provide a comparison vs Baseline of the logarithmic transformation of absolute ^{13}C-urea $AUC_{0\text{-}240min}$ for each DTX301 dose. Added the geometric mean, CV%, and geometric CV% for absolute ^{13}C-urea $AUC_{0\text{-}240min}$ and percent of normal absolute ^{13}C-urea $AUC_{0\text{-}240min}$ by visit. Added the mean and standard deviation for the logarithmic transformation of absolute ^{13}C-urea $AUC_{0\text{-}240min}$ and percent of normal absolute ^{13}C-urea $AUC_{0\text{-}240min}$. Updated the list of figures to be generated. • Section 8.3.2, Hyperammonemic Crises: Revised summary statistics to be generated. The number of HACs and the number of patients with HAC will be summarized by DTX301 dose, overall and by occurrence period (predose vs postdose). Predose HAC is defined as a HAC that occurred within the 12 months before dosing. • Section 8.3.3, 24-Hour Urinary Orotic Acid Excretion and Spot Urinary Orotic Acid: Urinary orotic acid $AUC_{0\text{-}24hr}$ and time-normalized urinary orotic acid $AUC_{0\text{-}24hr}$ will be derived following the same rules defined for plasma ammonia $AUC_{0\text{-}24hr}$ and time-normalized plasma ammonia $AUC_{0\text{-}24hr}$. These parameters will be listed. Spot urinary orotic acid will be listed. • Sections 8.3.4, Neutralizing Antibodies to AAV8; Section 8.3.5, AAV8 Binding Antibodies; Section 8.3.6, Cell-mediated Immune Response to AAV8 and OTC: Removed summary table. Clarified that observed results and change from Baseline to each scheduled postbaseline time point will be presented for subjects in the Safety Set in a listing. • Section 8.3.7, Anti-OTC Antibodies: Added that results will be presented for subjects in the Safety set in a listing. • Section 8.3.8, Ammonia Scavenger Therapy: Removed summary table. Added that results will be presented in a listing. • Section 8.3.9, Dietary Protein Prescription: Added that details of dietary protein prescription for each subject will be presented in a listing. • Section 8.3.10.1, Vocabulary and Matrix Reasoning Subtests from the WASI-II: Removed listing of raw and scaled scores of the Vocabulary and Matrix Reasoning subtests and Full Scale IQ from the WASI-II based on the Safety Set. • Section 8.3.11, PROMIS Mental Health Measures: Removed summary of observed values and change from Baseline for PROMIS Mental Health Measures. • Section 8.3.13: Subgroup Analysis: Added subgroup analysis plots by gender. • Section 9, Safety Analysis: Updated the list of safety parameters. • Section 9.1, Adverse Events: Added that any events that occur after exposure to DTX301 and before the first visit of Study 301OTC02 will be considered TEAEs. For summaries by CTCAE grade, removed that AEs missing a CTCAE grade will be considered CTCAE Grade 3. Added that the Investigator will assess each AE for relationship to [1-^{13}C]sodium acetate, OTC deficiency, hyperammonemia, and as of Protocol Amendment 5, corticosteroid regimen. Added a definition for HAC-related TEAE. Updated the AE summaries and listings to be generated. • Section 9.2, Death: Removed autopsy performed status because the data are not collected in eCRF. • Section 9.3, Clinical Laboratory Evaluations: Added spaghetti plots for ALT and AST levels over time. Added a box plot for ALT levels before and after DTX301 administration. Removed listing for ALT level and association with corticosteroid administration.

	• Section 10, Statistical Considerations for Data Monitoring Committee Meeting: Updated timing of planned DMC data review meetings. • Section 11, Interim Analysis: Updated the details regarding a possible interim analysis. Added that no interim analysis was conducted. • Section 12, Changes from Analyses Planned in the Protocol: Removed details regarding rules for deriving baseline values for the rate of ureagenesis. Added potential exploratory analyses of time-normalized plasma ammonia and/or percent of normal ureagenesis activity by gender. Updated timing of planned DMC data review meetings. Added that no interim analysis was conducted. Added that the N-CRM model will be run after subjects in Cohort 4 complete Week 12 for submission to Health Canada. Added mixed models to compare the logarithmic transformation of plasma ammonia $AUC_{0\text{-}24hr}$ and absolute ^{13}C-urea $AUC_{0\text{-}240min}$ vs Baseline. Added additional summary statistics for specific ammonia and ureagenesis parameters. • Section 13, References: Updated list of references.
Version 4.0 (25 November 2021)	• Section 3.1, Overall Study Design and Plan: Updated the priority of enrollment for Cohort 4. • Section 8.1, Rate of Ureagenesis: Removed OTC genotype from the fixed effects in the MMRM model. • Section 8.2, Plasma Ammonia Area Under the Curve from Time 0 to 24 Hours: Geometric summary statistics will be provided overall, by cohort, and for Cohorts 3 and 4 combined. A linear MMRM will provide a comparison of the logarithmic transformation of plasma ammonia $AUC_{0\text{-}24hr}$ for each DTX301 dose vs the lowest dose and at each visit vs Baseline. Removed OTC genotype from the fixed effects and baseline results as a continuous covariate in the MMRM model. • Section 8.3.8, Baseline Disease Management: Added definitions of Responder and Complete Responder for future development. • Section 8.3.9, Dietary Protein Prescription: In addition to total daily dietary protein intake, intact food protein and medical food protein will be summarized. Counts and percentages of subjects with type of change and of subjects on unrestricted protein diet will be generated. • Section 9.4, DTX301 Vector Genome Determination and Viral Shedding: Baseline value, highest value/timepoint, and timepoint of third consecutive negative results will be provided for each subject and sample type. Median values over time by cohort and sample type will be presented in a figure. Longitudinal displays over time by cohort for each subject and sample type will be generated. • Section 13, References: Updated list of references.

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List of Abbreviations

AAV8	adeno-associated virus serotype 8
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATC	anatomical therapeutic chemical
AUC	Area under the curve
BUN	blood urea nitrogen
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
C_{max}	maximum concentration
COVID-19	Coronavirus Disease 2019
CRM	continual reassessment method
CTMS	clinical trial management system
CV	Coefficient of Variation
[redacted]	[redacted]
DLT	dose limiting toxicity
DMC	data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report forms
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot assay
FACTS	Fixed and Adaptive Clinical Trial Simulator
GC	genome copies
GGT	gamma-glutamyl transferase
HAC	hyperammonemic crises
ICF	informed consent form
ICH	International Council for Harmonisation
IgG	immunoglobulin G
INR	international normalized ratio
IQ	intelligence quotient
IV	intravenous
LDH	lactate dehydrogenase
LLOQ	lower limit of quantification
LS	Least Squares
MCH	erythrocyte mean corpuscular hemoglobin
MCHC	erythrocyte mean corpuscular hemoglobin concentration
MCV	erythrocyte mean corpuscular volume

MedDRA	Medical Dictionary for Regulatory Activities
MMRM	mixed model for repeated measures
MTD	maximum tolerated dose
N-CRM	Neuenschwander's continual reassessment method
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
Neuro-QOL	Quality of Life in Neurological Disorders
OBD	optimal biological dose
OTC	ornithine transcarbamylase
PROMIS	Patient-Reported Outcomes Measurement Information System®
PT	preferred term
QTcB	QTc using Bazett's Correction
QTcF	QTc using Fridericia's Correction
RDW	erythrocytes, erythrocytes distribution width
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SE	standard error
SOC	system organ class
SS	safety set
TEAE	treatment-emergent adverse event
T_{max}	time to reach maximum concentration
UCD	urea cycle disorder
UCDC	Urea Cycle Disorders Consortium
UK	United Kingdom
ULN	upper limit of normal
ULOQ	upper limit of quantification
USA	United States of America
WAIS-IV	Weschler Adult Intelligence Scale, Fourth Edition
WASI-II	Weschler Abbreviated Scale of Intelligence, Second Edition
WHO	World Health Organization
WMI	Working Memory Index

1. Introduction

Ornithine transcarbamylase (OTC) deficiency, the most common of the urea cycle disorders (UCDs), is currently estimated to occur in up to 1:62,000 live births [Lichter-Konecki 2013]. OTC 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. OTC deficiency presents as a severe form in males and rarely in females shortly after birth (neonatal onset; ≤ 30 days of age) or later in life for 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].

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 (HAC) [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, prevention of HAC, and risk of neurological damage associated with OTC deficiency.

This statistical analysis plan (SAP) is based on the clinical study protocol 301OTC01, Amendment 5 dated 25th of February 2020 and its associated electronic case report forms (eCRF). This document describes the rules and conventions to be used in the dose selection, analysis and representation of safety and efficacy data as presented in the clinical protocol.

2. Study Objectives and Endpoints

The study objectives and endpoints are presented in Table 2-1.

Table 2–1 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 plasma 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 proteins after IV administration 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 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. Overall Study Design and Plan

Study 301OTC01 is a Phase 1/2, open-label, single arm, 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 optimal biological dose (OBD) for DTX301. The primary objective of the study is to determine the safety of single doses of DTX301. The secondary objectives are the assessment of rate of ureagenesis, reflecting the direct *in vivo* efficiency of the urea cycle and the assessment of plasma ammonia (area under the curve from 0 hours to 24 hours [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 as assessed by the ureagenesis assay employed [Matthews 1984; Jahoor 1987; Castillo 1996; Tuchman 2008b].

Eligible subjects will receive a single peripheral intravenous (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 drug. 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. Following the collection of a minimum of 12 weeks safety data, the data monitoring committee (DMC) will perform a 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 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 the OBD. Cohort 4 was prioritized in enrollment as OBD had been declared with cohort 3. Enrollment in cohort 4 was concluded after 2 patients enrolled as data on performance of prophylactic regimen, the focus of cohort 4, was evaluated and deemed sufficient.

3.2. Implementation of the N-CRM model

The purpose of this section is to define the proposed Neuenschwander's continual reassessment method (N-CRM) model for this study and to provide the details necessary to allow its implementation.

Where discrepancies exist between the text of this SAP and the study protocol document, the text in this SAP will prevail.

3.2.1. Candidate doses

The candidate doses to be considered are as follows:

Table 2 Candidate dose levels

Candidate Dosing Level	DTX301 Dose (GC/kg)
1	2×10^{12}
2	6×10^{12}
3	1×10^{13}
GC=genome copies	

3.2.2. Definition of a Dose Limiting Toxicity

A dose limiting toxicity (DLT) is defined as any Adverse Event (AE), with a toxicity grade of three or higher (AE ≥ Grade 3), that is considered related to the study drug by the investigator. As the N-CRM will be performed after 12 weeks of data have accrued for the last open cohort, only DLTs that occur within 12 weeks will be considered.

3.2.3. Definition of the Maximum Tolerated Dose

The estimate of the maximum tolerated dose (MTD) will be that dose with p(DLT) nearest to the target toxicity rate, regardless of whether or not the estimated p(DLT) is above or below the target toxicity rate.

3.2.4. Target toxicity rate

The target toxicity rate is 0.25. (*Nota Bene*: unlike the standard N-CRM model, this study will target a specific toxicity *rate* rather than a toxicity *band*.)

3.2.5. Escalation rule

The first cohort will receive $2x10^{12}$ GC/kg. A dose is cleared once a single cohort has been assessed at that dose. The maximum permitted dose escalation is 1 level relative to the highest cleared dose. Cohort 4 is not part of the cohorts included in the dose-escalation assessment as it is to be treated with the OBD defined with the assessment of the three previous dose cohorts.

3.2.6. N-CRM Stopping rules

The study will stop for success once results from six subjects treated at the current estimate of the MTD are available. Cohort 4 is not part of the assessment of the dose finding.
The CRM model will recommend that the study is stopped for insufficient safety if the current estimate of the MTD is $<2\times10^{12}$ GC/kg.

3.2.7. Dose-toxicity model

The relationship between dose and toxicity will be modelled using logistic regression with median dose as the reference dose and pseudo-dose levels (the x-hats) defined as log(dose/reference). The minimum and maximum values for p(DLT) are 0 and 1 respectively.

Thus, we have

$$p(DLT|\hat{x},\alpha,\beta) = \frac{e^{\alpha+\beta\hat{x}}}{1+e^{\alpha+\beta\hat{x}}}$$

Where the joint prior for α and β is given by

$$\begin{pmatrix}\alpha \\ \log\beta\end{pmatrix} \sim N\left(\begin{pmatrix}\mu_\alpha \\ \mu_\beta\end{pmatrix}, \begin{pmatrix}\sigma_\alpha \times \sigma_\alpha & \sigma_\alpha \times \sigma_\beta \times \rho \\ \sigma_\alpha \times \sigma_\beta \times \rho & \sigma_\beta \times \sigma_\beta\end{pmatrix}\right)$$

Initial values for μ_α, μ_β, σ_α, σ_β and ρ are based on the following assumptions for the distribution of p(DLT) at each dose:

Dose	2.5% centile	Median (50% centile)	97.5% centile
$2x10^{12}$ GC/kg	0.006	0.03	0.65
$6x10^{12}$ GC/kg	0.008	0.06	0.70
$1x10^{13}$ GC/kg	0.012	0.12	0.80

which lead to the following parameterization of the joint prior:

Alpha		Log(Beta)		
Mean	SD	Mean	SD	Rho
-2.3097	1.6604	-0.4323	0.0044	-0.2405

Thus, we set μ_α= -2.3097, logμ_β = -0.4323, σ_α = 1.6604, logσ_β = 0.0044 and ρ = -0.2405.

Since the standard deviation (SD) of α is non-zero, the intercept is allowed to vary.

3.2.8. Number of toxicities required before all doses are considered unsafe

The CRM model will not recommend stopping for insufficient safety until at least two DLTs have been observed. Note: this affects only the dose recommendation provided by the N-CRM model. It does not prevent an earlier termination based on clinical review of data emerging from the study.

3.2.9. Cohort size

The cohort size will be three evaluable patients in every case for cohorts 1 to 3 as the expansion of cohort 3 and cohort 4 is not part of the N-CRM assessment. A patient is classed as evaluable if the patient remained in the study for at least 12 weeks after dosing or a DLT was observed within 12 weeks of dosing. Should a protocol amendment change the inclusion / exclusion criteria of the study for safety, then a decision will be taken and documented on which patients to include in the CRM model to recommend the next dose after the study resumes. The N-CRM model will not be modified to take into account any protocol amendment that may be developed after the first N-CRM model has been run.

3.2.10. Other clarifications

- The N-CRM model will not contribute to the identification of the OBD. Estimation of the OBD will not influence the dose recommended by the CRM, but may be taken into account by the DMC when making the dosing decision for the next cohort.
- A DMC recommendation to administer a dose other than the three doses listed in section 3.2.1 above will be implemented only after a protocol amendment.

3.3. Treatments

Subjects will receive a single, peripheral IV infusion of DTX301, administered by qualified study personnel as designated by the investigator. The dose will be determined by the cohort and candidate dose.

Subjects in cohort 4 will be treated with the OBD defined with the assessment of the three previous dose cohorts. Subjects treated in cohort 4 (Dosing Process Optimization) will utilize an alternative regimen of corticosteroids, aiming at prophylaxis of vector-induced hepatitis. Therefore, prednisone (or prednisolone) will be initiated before dosing with DTX301, sustained for 4 weeks, followed by tapering.

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 weight at Screening.

The efficacy data will be modeled by dose (i.e. cohorts 3 and 4 will be combined, with OBD being determined as $1.0x10^{13}$ GC/kg). The other data will be summarized/reported by DTX301 dose/prophylactic corticosteroids (i.e. by cohort) and presented in the tables, figures and listings as follows:

- DTX301 $2.0x10^{12}$ GC/kg
- DTX301 $6.0x10^{12}$ GC/kg
- DTX301 $1.0x10^{13}$ GC/kg
- DTX301 $1.0x10^{13}$ GC/kg + Prophylactic corticosteroids

4. General Statistical Considerations

4.1. Baseline and Change from Baseline

4.1.1. Ureagenesis Baseline

Samples for ureagenesis are collected over a 4 hour-period at Screening and Day 1 to assess baseline ureagenesis. The screening assessment can be repeated if discrepant with subject's clinical status and severity.
Baseline of ureagenesis is defined as the average of Screening and Day 1 results. If the absolute difference of results between Screening and Day 1 is ≥ 25% of normal rate of ureagenesis, then the Screening value will become the Baseline for ureagenesis.
If the Screening assessment has been repeated (i.e. unscheduled assessment) then the results from the unscheduled Screening visit will be used in lieu of the Screening results to determine the Baseline of ureagenesis.
This rule will be used to determine the visits to be included in the baseline derivation for each of the ureagenesis parameters (derived and reported).

For the non-AUC supplementary ureagenesis parameters, a baseline value is defined [redacted]

For $AUC_{0\text{-}240min}$ of absolute ^{13}C-urea and $AUC_{0\text{-}240min}$ of ^{13}C-Bicarbonate, the baseline value is defined [redacted] For percent of normal $AUC_{0\text{-}240min}$ of absolute ^{13}C-urea, [redacted]

4.1.2. Other Parameters Baseline

For spot ammonia, plasma ammonia $AUC_{0\text{-}24h}$ and urinary orotic acid $AUC_{0\text{-}24h}$ the baseline value is defined [redacted]. For urinary orotic acid, a baseline value is defined [redacted] For ammonia scavenger therapy usage, the baseline value is defined [redacted] For all other endpoints, the baseline value is [redacted]

4.1.3. Change from Baseline

All explicit references to change from baseline in this SAP refer to absolute change from baseline. Change from baseline is defined [redacted] Percent change from baseline is defined [redacted] If either the baseline or post-baseline value is missing, then the change from baseline and percent change from

baseline value are set to missing. For each time point where change from baseline is evaluated for continuous variables, descriptive statistics will be displayed for the values at baseline, values at the given time point, and values for change from baseline at the given time point for those subjects who have data at both the baseline and the time point being evaluated.

4.2. Conversion of Days to Weeks or Months

The following conversion factors will be used to convert days into weeks or months: 1 week = 7 days, 1 month = 30.4375 days.

4.3. Duration

Duration will be calculated as stop date – start date + 1, unless otherwise specified. Duration is calculated only when both dates are complete. Examples include duration of study participation, AE duration, and duration of oral steroid treatment.

4.4. Missing Data

Refer to the Appendices for imputation rules for partial and missing AE onset dates as well as partial and missing prior/concomitant medication start and end dates. For the adeno-associated virus serotype 8 (AAV8) binding antibody immunoglobulin (IgG), neutralizing antibodies to AAV8, amino acid panel, cell-mediated immune Response to AAV8 and OTC, chemistry, hematology (including coagulation panel), plasma ammonia (24 hours and spot), ureagenesis assay parameters, urinalysis, urinary orotic acid, vector genome determination (blood) and viral shedding (urine, saliva, and stool) laboratory data, quantitative results either below the lower limit of quantification (LLOQ) or above the upper limit of quantification (ULOQ) will be set to the LLOQ or ULOQ, respectively. Otherwise, no imputation of partial or missing values will be performed.

4.5. Multiple Testing

No formal adjustment for multiplicity will be performed.

4.6. Output Numbering

Numbering for tables, listings and figures will follow International Council for Harmonisation (ICH) E3 Guidelines.

4.7. Presentation of Continuous and Categorical Variables

Continuous data will be summarized using descriptive statistics (i.e., n, arithmetic mean, SD, median, minimum, and maximum). Categorical data will be summarized using event/subject count and percentage (n, %) in each category. Percentages will generally be reported to one decimal place. For descriptive statistics of all numeric variables, unless otherwise specified, the minimum and maximum will be displayed to the same level of precision as reported. Mean and median will be displayed to one level of precision greater than the data collected. SD will be displayed to two levels of precision greater than the data collected. P-values will be rounded to three decimal places. If a p-value is less than 0.001,

it will be reported as "<0.001." If a p-value is greater than 0.999, it will be reported as ">0.999."

When count data are presented, the percentage will be suppressed when the count is zero in order to draw attention to the non-zero counts. A row denoted "Missing" will be included in count tabulations where specified in the shells to account for dropouts and missing values. The denominator for all percentages will be the number of subjects within the analysis set of interest, unless otherwise specified.

4.8. Randomization, Stratification, and Blinding

This is an open label study, so there is no blinding of DTX301 dose, stratification, or randomization.

4.9. Sample Size

The study is expected to enroll up to 18 subjects. Three subjects will be enrolled into cohort 1 and then sequentially into cohorts of a minimum of 2 to 3 subjects. This sample size consideration is consistent with the sample size of other studies of similar design but is not based on a power calculation.

4.10. Significance Level

Unless otherwise specified, all testing of statistical significance will be 2-sided, and all p-values less than or equal to 0.05 will be considered statistically significant. Similarly, all confidence intervals (CIs) will be two-sided with a significance level of 5%.

4.11. Software

Modelling to determine the OBD will be conducted [redacted] All other general data manipulation and statistical analyses will be conducted using SAS® Version 9.4 or higher (SAS® Institute, Inc., Cary, North Carolina, United States).

4.12. Study Day and Study Start Date

For assessments prior to DTX301 administration, study day will be defined as the assessment/event date minus date of DTX301 infusion (Day 1). For assessments on or after Day 1, study day will be defined as assessment/event date minus date of DTX301 infusion + 1. Study start date is defined as the date the informed consent form (ICF) is signed.

4.13. Subject Identification

Subjects will be identified in data listings by subject identification number which consists of the subject number concatenated with the site number. Data listings will be sorted by subject identification number, visit, and parameter (where applicable).

4.14. Unscheduled Visits

Unscheduled visits will not be included in by-visit summaries of data with the exception of worst post-baseline value in the shift from baseline summary of electrocardiogram (ECG) interpretation and summaries of worst post-baseline value for hematology, chemistry, urinalysis and amino-acid and summaries of newly notable laboratory abnormalities in chemistry relating to liver function test results. Moreover, unscheduled visits will be included into individual patient graphs.

4.15. Analysis Sets

4.15.1. Safety Set

The Safety Set (SS) will include all subjects who receive DTX301. Subjects who receive a partial dose will be summarized or listed according to the nearest candidate dose (see Table 3-1) based on the actual dose they received. The SS will be used to tabulate and list all of the safety endpoints, efficacy endpoints, and for the purpose of N-CRM modelling.

4.15.2. MTD Determining Set

See Section 3.2.8 for details.

5. Subject Disposition

5.1. COVID-19

At the onset of the Coronavirus Disease 2019 (COVID-19) pandemic in Europe and North America, only 2 subjects in cohort 3 were ongoing [redacted] Due to the spacing of visits and number of ongoing subjects, it was decided to collect the impact of COVID-19 on the visits and/or assessments missed using a specific eCRF page and not to change the planned analysis. This data collection method was planned to be applied to patient [redacted] and, if applicable, to all patients of cohort 4 as well. All data collected on this page will be listed in a data listing.

Table 5–1 Pandemic Lock-down Restrictions Start/End Dates

Country (region)	Lock-down Start Date	Lock-down Stop Date
UK	24-Mar-2020	15-Jun-2020
	05-Nov-2020	02-Dec-2020
	04-Jan-2021	12-Apr-2021
Spain	14-Mar-2020	11-May-2020
France*	17-Mar-2020	11-May-2020
	30-Oct-2020	14-Dec-2020
Canada	13-Mar-2020	04-May-2020

USA (Massachusetts)	24-Mar-2020	25-May-2020
USA (New York)	20-Mar-2020	10-June-20

The start and stop dates correspond to the lock-down in each country/region, travel restriction may have been lifted at a later date in some country/regions.

[redacted].

5.2. Disposition

Disposition will be summarized for all enrolled subjects (i.e. all subjects who signed the study informed consent and met all Screening Inclusion Criteria and did not meet any Screening Exclusion Criteria) by DTX301 dose/prophylactic corticosteroids and overall. The number and percentage of subjects who have been treated (i.e. SS), have completed the study, are ongoing or discontinued from the study, as well as the primary reason for study discontinuation (if applicable) will be tabulated.
All disposition related information for enrolled subjects will be presented in a data listing.

5.3. Protocol Deviations

Protocol deviations will be recorded within the PPD Clinical Trial Management System (CTMS) and undergo cross-functional team review prior to database lock. The Deviation Guidance Document contains all potential protocol deviations, classified by CTMS subtype. In addition, protocol deviation classification (i.e. significant vs. not significant) as determined by Ultragenyx Pharmaceutical Inc. prior to database lock will be documented in CTMS. All protocol deviations will be presented in a data listing for the SS.

5.4. Duration of Study Participation

The duration of study participation will be summarized by DTX301 dose/prophylactic corticosteroids for all subjects based on the SS. The duration of study participation (days) is calculated as the date of the last visit (as recorded on the Study Completion/Early Withdrawal page) minus date of the first dose plus 1. If the date of the last visit on the Study Completion/Early Withdrawal eCRF page is missing or if a subject is lost to follow-up, the latest available visit date will be used. The number of subjects in each of the following study participation duration categories will be displayed: ≤ 12 weeks (84 days), > 12 – 24 weeks (85 to 168 days), > 24 – 36 weeks (169 to 252 days), > 36 – 52 weeks (253 to 364 days), and > 52 weeks (365 days).

6. Demographics and Baseline Characteristics

6.1. Demographics

Baseline demographics will be summarized by DTX301 dose/prophylactic corticosteroids and overall for all subjects in the SS. Baseline demographic data to be evaluated will include age, sex, race, ethnicity, height, and screening and baseline weight. The number of subjects in each of the following age categories will be displayed: 18 - < 30 years, 30 - < 40 years, 40 - < 50 years, 50 - < 60 years, ≥ 60 years.

Descriptive statistics will be presented for age, height and weight. Age category, sex, race, and ethnicity will be summarized categorically.
All demographic data and baseline characteristics, including contraception, for subjects in the SS will be listed.

6.2. Medical History

Medical history will be summarized by DTX301 dose/prophylactic corticosteroids for all subjects in the SS. Medical history data will be summarized by DTX301 dose/prophylactic corticosteroids and overall and by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term (PT). Medical history data will be sorted in alphabetical order by SOC; PTs will be sorted in descending order by frequency within each SOC.
Medical history data will be also listed. The dictionary version used for reporting the study will be described in the relevant table and listing footnotes.

6.3. OTC Deficiency Medical History

OTC deficiency medical history will be summarized by DTX301 dose/prophylactic corticosteroids for all subjects in the SS. The following OTC deficiency medical history items will be summarized:

- Time since initial diagnosis of OTC deficiency (years)
- Time since subject started therapy for OTC deficiency (years)
- Family history of OTC deficiency (Yes/No)
- OTC deficiency currently stable (Yes/No)
- Time since OTC deficiency became stable (weeks)
- Currently on stable dose of ammonia scavenger therapy (Yes/No)
- Time since stable dose of ammonia scavenger therapy (weeks)

The date of initial diagnosis of OTC deficiency and date the subject started therapy for OTC deficiency will be imputed as described in the Appendices. All time-related items will be calculated as the time from the date of interest to the date of Screening, inclusive.
A subject data listing will be provided with all OTC deficiency medical history data for the SS.

6.4. OTC Genotyping

A listing will be provided with OTC genotype results for the SS.

6.5. Inclusion and Exclusion Criteria

All inclusion/exclusion criteria related information for enrolled subjects will be presented in a data listing. The listing will include the patients who failed inclusion/exclusion criteria.

7. Treatments and Medications

7.1. Prior and Concomitant Medications

Medications will be coded using the World Health Organization (WHO) Drug Dictionary. The WHO Drug Dictionary version used for reporting the study will be described in the relevant table and listing footnotes. If the medication start or end date is missing, it will be imputed before summary as described in the Appendices. If the start date of a medication is completely missing and the end date is before dosing of study drug on Day 1, it will be counted as a prior medication. If the start date of a medication is completely missing and the end date is after dosing of study drug on Day 1, it will be counted as both a prior and concomitant medication. If the start date of a medication is on or after the dose of study drug on Day 1 and the end date of the medication is completely missing, it will be counted as a concomitant medication.
A subject data listing will be provided with medications data for the SS; a flag will be included to identify prior vs. concomitant medications.

7.1.1. Prior Medications

Prior medications are defined as those medications with a recorded end date before dosing of study drug on Day 1. The total number of prior medications will be summarized. The number and percentages of subjects with at least one prior medication will be summarized overall and by Anatomical Therapeutic Chemical (ATC) level 4 term and PT. Prior medications will be presented by DTX301 dose/prophylactic corticosteroids and overall using the SS. Medications will be sorted in descending order by ATC level 4 term based on counts in the overall column; PTs will be sorted in descending order of frequency within each ATC level 4 term based on counts in the overall column.

7.1.2. Concomitant Medications

Concomitant medications are defined as those medications that are taken on or after dosing of study drug on Day 1.

The total number of concomitant medications will be summarized. The number and percentages of subjects with at least one concomitant medication will be summarized by ATC level 4 term and PT. Concomitant medications will be presented by DTX301 dose/prophylactic corticosteroids and overall using the SS. Medications will be sorted in descending order by ATC level 4 term based on counts in the overall column; PTs will be sorted in descending order of frequency within each ATC based on counts in the overall column.

7.2. Study Drug

The following details related to study drug administration on Day 1 will be listed for each subject in the SS:

- Date of infusion

- Start time and End time
- Planned dose
- Dose administered
- Dose interrupted status (Yes/No) and associated reason
- Total volume infused
- Dose adjusted status (Yes/No) and associated reason
- Anatomical location of administration

7.3. [1-^{13}C] sodium acetate

Details related to [1-^{13}C] sodium acetate administration will be listed for each subject in the SS.

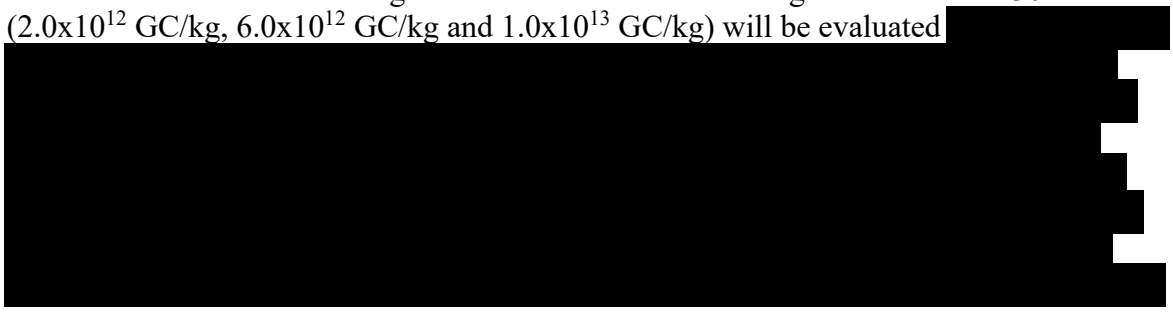
8. Efficacy Analysis

8.1. Rate of Ureagenesis

This efficacy endpoint is defined as the change from baseline in rate of ureagenesis measured by the generation of ^{13}C-urea over 4 hours as determined by isotope ratio mass spectrometry at Weeks 6, 12, 20, 24, Early Withdrawal, and End of Study (Week 52). The observed, change from baseline, and percent change from baseline in rate of ureagenesis will be summarized by DTX301 dose/prophylactic corticosteroids and visit based on the SS.

Rate of ureagenesis (µmol*h/kg) for a given patient at a specific visit will be estimated using the following steps:

The association between change from baseline in rate of ureagenesis and DTX301 dose ($2.0x10^{12}$ GC/kg, $6.0x10^{12}$ GC/kg and $1.0x10^{13}$ GC/kg) will be evaluated



[redacted]

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

This efficacy endpoint is defined as change from baseline in the $AUC_{0\text{-}24h}$ of plasma ammonia at Weeks 6, 12, 24, Early Withdrawal, and End of Study (Week 52). Two samples will be collected at each visit and timepoint. One sample will be processed by the local laboratory and one sample will be processed by the central laboratory. The observed, change from baseline, and percent change from baseline in the $AUC_{0\text{-}24h}$ of plasma ammonia will be summarized by category (i.e. central laboratory vs. local laboratory), DTX301 dose/prophylactic corticosteroids , and visit based on the SS. Change from baseline in the $AUC_{0\text{-}24h}$ of plasma ammonia will be analyzed [redacted] used for change from baseline in rate of ureagenesis based on the SS.

The baseline for the $AUC_{0\text{-}24h}$ measurement is based on the hourly plasma ammonia results beginning on Day 0 and ending on Day 1 prior to DTX301 administration. $AUC_{0\text{-}24h}$ will be calculated [redacted] The rules for handling of missing data for the $AUC_{0\text{-}24h}$ calculation are described in the Appendices.

The plasma ammonia listing and summary will also include the time-normalized $AUC_{0\text{-}24h}$, calculated [redacted]

[redacted]

The following additional summary statistics will be provided (overall, by DTX301 dose/prophylactic corticosteroids and for Cohorts 3 and 4 combined) for both $AUC_{0\text{-}24h}$ and time-normalized $AUC_{0\text{-}24h}$ of plasma ammonia by visit: geometric mean, coefficient of variation (CV) %, geometric CV%. The mean and the standard deviation will also be provided for the logarithmic transformation of both $AUC_{0\text{-}24h}$ and time-normalized $AUC_{0\text{-}24h}$ of plasma ammonia.

An additional linear mixed model for repeated measures will provide a comparison of the [redacted]

A longitudinal plot of plasma ammonia (as determined by central lab) will be provided by subject, including the display of the $AUC_{0\text{-}24h}$ for the subject alongside each plot.

A longitudinal plot of plasma ammonia $AUC_{0\text{-}24h}$ (as determined by central lab), alanine aminotransferase (ALT) level (as determined by central lab for both scheduled and unscheduled visits), and corticosteroid regimen (prednisone/prednisolone) over time (each dose taken at the visit and possibly dose modifications in-between) will also be provided by subject.

A plot displaying the rate of ureagenesis percentage of normal, the time-normalized plasma ammonia $AUC_{0\text{-}24h}$ (as determined by central laboratory) and corticosteroid regimen (prednisone/prednisolone) over time (each dose taken at the visit and possibly dose modifications in-between) will be provided by subject.

A panel plot displaying the time-normalized plasma ammonia $AUC_{0\text{-}24h}$ (as determined by central laboratory), spot ammonia (as determined by local lab), ALT level (as determined by central lab for both scheduled and unscheduled visits) and corticosteroid

regimen (prednisone/prednisolone) over time (each dose taken at the visit and possibly dose modifications in-between) will be provided by subject.

Results from the spot ammonia samples collected as part of clinical chemistry will be reported alongside the 24-hour ammonia sample results and its derived parameters.

8.3. Exploratory Endpoints

8.3.1. Supplementary Ureagenesis Parameters

The observed, change from baseline, and percent change from baseline for $AUC_{0\text{-}240min}$ of absolute ^{13}C-urea, percent of normal $AUC_{0\text{-}240min}$ of absolute ^{13}C-urea, $AUC_{0\text{-}240min}$ of ^{13}C-bicarbonate, and ^{13}C-ureagenesis assay measures (e.g. total plasma urea [mmol/L], enrichment of ^{13}C-bicarbonate (atom % excess), enrichment of ^{13}C-urea [atom % excess], absolute ^{13}C-urea concentration [µmol/L], C_{max} 0-240min absolute ^{13}C-urea [µmol/L], and T_{max} 0-240min absolute ^{13}C-urea [min]) will also be summarized by DTX301 dose/prophylactic corticosteroids, visit at Weeks 6, 12, 20, 24, Early Withdrawal, and End of Study (Week 52), and time point (0, 1, 1.5, 2, 3, and 4 hours relative to dosing with [1-^{13}C]sodium acetate) where applicable based on the SS.

[redacted]

The following additional summary statistics will be provided (overall, by DTX301 dose/prophylactic corticosteroids and for Cohorts 3 and 4 combined) for $AUC_{0\text{-}240min}$ of absolute ^{13}C-urea and percent of normal $AUC_{0\text{-}240min}$ of absolute ^{13}C-urea by visit: [redacted] The mean and the standard deviation will also be provided [redacted]

8.3.2. Hyperammonemic crises (HAC)

Following protocol amendment 5, HACs are specifically collected under the definition of the UCDCas 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].

The number of HACs as well as the number of patients with HAC will be summarized by DTX301 dose/prophylactic corticosteroids, both overall and by occurrence period (pre-dose vs. post-dose). Pre-dose hyperammonemic crises are defined as HACs occurred in the 12 months before dosing. No further breakdown by visit will be provided as it is unlikely a patient has more than one crisis during the study.

8.3.3. 24-hour Urinary Orotic Acid Excretion and Spot Urinary Orotic Acid

The observed, change from baseline, and percent change from baseline in 24-hour urinary orotic acid excretion will be summarized by DTX301 dose/prophylactic corticosteroids , visit at Weeks 6, 12, 24, Early Withdrawal, and End of Study (Week 52), and time point (0, 6, 12, 18, and 24 hours relative to the start of plasma ammonia determination) based on the SS. Change from baseline and percent change from baseline will be calculated by matching time point (e.g. 0 hour results at each post-baseline visit will be compared to the 0 hour result at baseline).

In addition, Urinary Orotic Acid $AUC_{0\text{-}24h}$ and its time-normalized version will be derived [redacted] These parameters will only be displayed in the listing.

Spot Urinary Orotic Acid will be listed only.

8.3.4. Neutralizing Antibodies to AAV8

Samples for neutralizing antibodies to AAV8 will be collected at scheduled time points to monitor for a humoral immune response to AAV8. Observed results and change from baseline to each scheduled post-baseline time point will be presented for each subject in the SS in a listing. Observed results will also be presented over time along with AAV8 binding antibody IgG results in a panel plot for each subject.

From protocol amendment 5, AAV8 neutralizing antibody assessments were reduced based on medical consideration of results to date and to reduce subject burden.

8.3.5. AAV8 Binding Antibodies

Samples for AAV8 binding antibody IgG will be determined [redacted] Observed results and change from baseline to each scheduled post-baseline time point will be presented for each subject in the SS in a listing. Observed results will also be presented over time along with AAV8 neutralizing antibody results in a panel plot for each subject.

From protocol amendment 5, AAV8 binding antibody assessments were reduced based on medical consideration of results to date and to reduce subject burden.

8.3.6. Cell-mediated Immune Response to AAV8 and OTC

The presence of T-cell specific response for AAV8 and OTC will be determined by an enzyme-linked immunospot assay (ELISPOT) at scheduled time points. Observed results and change from baseline to each scheduled post-baseline time point will be presented for each subject in the SS in a listing. Observed results will also be presented over time in a panel plot, split by DTX301 dose/prophylactic corticosteroids and by subject.

Following protocol amendment 5, the assessment of 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; moreover, require notable blood volume to be collected from subjects.

The planned outputs will be still created, but patients enrolled under protocol amendment 5 or a subsequent protocol amendment will not be included.

8.3.7. Anti-OTC Antibodies

The presence of circulating anti-OTC antibodies will be determined by ELISA at scheduled time points. The number and percentage of subjects will be presented by result category (i.e. Positive vs. Negative) and DTX301 dose/prophylactic corticosteroids in the SS at each scheduled post-baseline time point.
Results (both categorical and continuous) will be displayed in a subject data listing for the SS.

8.3.8. Baseline disease management

Baseline disease management included ammonia-scavenging drugs and protein-restricted diet.
Responder: A patient who has 50% reduction in baseline disease management.
Complete responder: A patient who completely discontinues ammonia-scavenging drugs and protein-restricted diet.

8.3.8.1. Ammonia Scavenger Therapy

Ammonia scavenger therapy will be displayed in a subject data listing.

8.3.8.2. Dietary Protein Prescription

Total daily dose (g) of prescribed dietary protein intake, intact food protein and medical food protein will be summarized by DTX301 dose/prophylactic corticosteroids and visit based on the SS using the descriptive statistics. Counts and percentages of subjects with type of change will be summarized for total daily intact food protein and total daily medical food protein by DTX301 dose/prophylactic corticosteroids and visit.
Counts and percentages of patients on unrestricted protein diet (i.e. unlimited intact food assumption and no medical food administration) at each visit will be provided.

Details of dietary protein prescription for each subject will be presented in a subject data listing.

8.3.9. Neuropsychological Tests

The observed and change from baseline for Patient-Reported Outcomes Measurement Information System® (PROMIS®) mental health measurements and the [redacted] will be summarized by DTX301 dose/prophylactic corticosteroids and visit at Early Withdrawal and End of Study (Week 52) unless otherwise noted based on the SS. All scoring will be performed by the study site psychologist or supervised designee and reviewed for inconsistencies or potential inaccuracies by a psychologist working as a consultant for Ultragenyx (CORE division). Both raw and scaled/standardized scores will be listed by subject and test type based on the SS for each of the following neuropsychological tests.

8.3.9.1. Vocabulary and Matrix Reasoning Subtests from the WASI-II

Subjects will be asked to complete the Vocabulary and Matrix Reasoning subtests from the Weschler Abbreviated Scale of Intelligence, Second Edition (WASI-II) at the Baseline Visit only. The WASI-II provides a general measure of cognitive functioning in subjects aged 6 to 89 years. The Vocabulary and Matrix Reasoning subtests provide an accurate, validated assessment of intelligence quotient (IQ) that is highly correlated (r=0.86) with results from the Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV). Scaled scores for the Vocabulary and Matrix Reasoning subtests range from 20 to 80 whereas scaled scores for the Full Scale IQ range from 40 to 160.

8.3.9.2. [redacted]

8.3.9.3. [redacted]

8.3.9.4. [redacted]

8.3.10. PROMIS® Mental Health Measures

Subjects will be asked to complete PROMIS® measures consisting of Anxiety (Adult Short Form 8a) and Depression (Adult Short Form 8a) at Day 0 (pre-dose) and at Weeks 6, 12, 24, and End of Study (Week 52). Higher scores indicate more of the concept being measured. Each measure consists of 8 items ranging from 1 to 5. Each measure can be scored as long as the subject answered at least 4 items. The formula for creating the total raw (or pro-rated) score is shown below:

[redacted]

If the resulting total raw score is a fraction, round up to the nearest integer. Next, the total raw score is translated into a standardized score (T-score) using the applicable score conversion table (See Appendices). The T-score rescales the total raw score into a standardized score with a mean of 50 and a SD of 10. Therefore, a subject with a T-score of 40 is one SD below the mean. All scoring will be performed by the study site psychologist or supervised designee and reviewed for inconsistencies or potential inaccuracies by a psychologist working as a consultant for Ultragenyx (CORE division). All inconsistencies or potential inaccuracies will be queried and resolved appropriately prior to database lock.

8.3.11. Quality of Life in Neurological Disorders Measures

Subjects will be asked to complete the Quality of Life in Neurological Disorders (Neuro-QoL) Item Bank v2.0 measure of Cognitive Function – Short Form and Item Bank v1.0 measure of Emotional/Behavioral Dyscontrol – Short Form at Day 0 (predose) and at Weeks 6, 12, 24, and End of Study (Week 52). Higher scores indicate more of the concept being measured. Each measure consists of 8 items ranging from 1 to 5. Each measure can be scored as long as the subject answered at least 4 items. The formula for creating the total raw (or pro-rated) score is shown below:

[redacted]

If the resulting total raw score is a fraction, round up to the nearest integer. Next, the total raw score is translated into a standardized score (T-score) using the applicable score conversion table (See Appendices). The T-score rescales the total raw score into a standardized score with a mean of 50 and a SD of 10. Therefore, a subject with a T-score of 40 is one SD below the mean. All scoring will be performed by the study site psychologist or supervised designee and reviewed for inconsistencies or potential inaccuracies by the psychologist at Ultragenyx. All inconsistencies or potential inaccuracies will be queried and resolved appropriately prior to database lock.

8.3.12. Subgroup analysis

Graphical displays of time-normalized ammonia and percent of normal ureagenesis activity over time will be provided by gender.

9. Safety Analysis

Safety will be assessed based on AEs, serious adverse event (SAEs), complete and targeted physical examination findings, vital sign measurements, ECG results, clinical laboratory evaluations (clinical chemistry, spot ammonia [included under efficacy section], hematology, urinalysis, and coagulation panel), blood for vector genome determination, measurement of neutralizing antibody to AAV8, measurement of AAV8 binding antibodies, and anti-OTC antibodies [the latter three included under efficacy section]. Assessment of any cell-mediated immune responses to AAV8 and OTC are collected prior to amendment 5. All safety analyses will be conducted for the SS.

9.1. Adverse Events

An AE is defined as any untoward medical occurrence in a subject enrolled into this study (from the time the subject signs the ICF until their exit from the study) regardless of its causal relationship to study drug. A treatment-emergent adverse event (TEAE) is defined as any event not present before exposure to study drug, or any event already present that worsens in intensity (i.e. severity) or frequency after exposure to study drug. Any events which occur after exposure to study drug and before the first visit of the extension study (301OTC02) will also be considered as TEAEs.

AEs will be coded using MedDRA; the dictionary version used for reporting the study will be described in the relevant table and listing footnotes. Severity/toxicity grade will be defined according to the most current version of the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE).

There are four categories of relationship collected on the eCRF: “Unrelated”, “Possible”, “Probable”, or “Definite”. A related AE is an event where the investigator determined that the relationship to study drug was “Possible”, “Probable”, or “Definite”. For summaries by relationship, AEs with a missing relationship will be considered to be “Possible”. TEAEs will be summarized by strongest relationship. The investigator will also indicate whether

the AE is related to [1-^{13}C] sodium acetate, OTC deficiency, hyperammonemia, and as of protocol amendment 5, to corticosteroid regimen.

An HAC-related TEAE is defined as a TEAE which has been identified as associated with the crisis in the eCRF "Hyperammonemic Crisis" page.

If AE start date is missing, it will be imputed as described in the Appendices.

The incidence and the number and percentage of subjects will be summarized by DTX301 dose/prophylactic corticosteroids and overall for each of the following:

- All TEAEs
- All serious TEAEs
- All TEAEs with grade ≥3
- All study drug related TEAEs
- All study drug related serious TEAEs
- All study drug related TEAEs ≥ grade 3
- All TEAEs related to corticosteroid regimen (prednisone/prednisolone)
- All HAC-related TEAEs
- TEAEs leading to study discontinuation
- Any AE leading to death

The number and percentage of subjects with TEAEs will be summarized by DTX301 dose/prophylactic corticosteroids, overall, SOC, and PT for each of the following:

- TEAEs (including incidence)
- TEAEs by relationship (including incidence)
- TEAEs by severity (including incidence)
- TEAEs by severity and relationship
- TEAEs related to corticosteroid regimen (prednisone/prednisolone)
- HAC-related TEAEs
- Serious TEAEs
- DLTs

At each level of summarization, a subject will be counted only once for each TEAE he/she experiences within that level. Percentages will be calculated out of the number of subjects in the applicable analysis set. TEAEs will be sorted in alphabetical order by SOC. PTs will be sorted in descending order by frequency within each SOC.

A data listing will be provided in the SS for each of the following:

- All AEs
- All SAEs

- DLTs
- AEs leading to study discontinuation
- AEs related to corticosteroid regimen

9.2. Death

All subjects who have an AE with an outcome of death will be presented in a listing. Date of death, and death certificate completion status will be included.

9.3. Clinical Laboratory Evaluations

Summary tables presenting observed values and changes from baseline to each scheduled post-baseline time point and minimum/maximum post-baseline value will be provided for clinical chemistry, hematology, urinalysis, and amino acids using standardized results.

Results of clinical chemistry, hematology, and amino acid parameters will be categorized as low, normal, or high according to laboratory range specifications. Shifts from baseline to each scheduled post-baseline time point will be presented to show the number and percentage of subjects in each category by parameter.

Results of urinalysis parameters will be categorized as normal or abnormal. Shifts from baseline to each scheduled post-baseline time point will be presented to show the number and percentage of subjects in each category by parameter.

Spaghetti plots for both ALT and aspartate aminotransferase (AST) results separately, over time will be provided.
A box plot of ALT results before and after gene transfer will be provided.

Clinical Chemistry

- ALT, albumin, alkaline phosphatase (ALP), AST, bilirubin (total and indirect), blood urea nitrogen (BUN), calcium, carbon dioxide, chloride, creatine kinase, creatinine, gamma-glutamyl transferase (GGT), glucose, magnesium, lactate dehydrogenase (LDH), phosphate, potassium, sodium, and total protein

Hematology (including Coagulation Panel)

- activated partial thromboplastin time (aPTT), basophils, basophils/leukocytes, eosinophils, eosinophils/leukocytes, erythrocyte mean corpuscular hemoglobin (MCH), erythrocyte mean corpuscular hemoglobin concentration (MCHC), erythrocyte mean corpuscular volume (MCV), erythrocytes, hematocrit, hemoglobin, leukocytes, lymphocytes, lymphocytes/leukocytes, monocytes, monocytes/leukocytes, neutrophils, neutrophils/leukocytes, platelets, international normalized ratio (INR), prothrombin time.

Urinalysis

- blood, glucose, ketones, pH, protein, specific gravity, and microscopic examination (if blood or protein is found)

Amino Acid Panel

- Alanine, amino-butyric acid, arginine, asparagine, aspartic acid, , citrulline, cystine, ethanolamine, glutamic acid (aka "serum glutamate"), glutamine, glycine, histidine, homocysteine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, and valine.

Clinical chemistry measurements will be processed by both PPD Global Central Laboratory and local laboratories (liver function tests only) and summarized separately. Hematology and urinalysis measurements will be processed only by PPD Global Central Laboratory and summaries related to these laboratory categories will be limited to central lab results.

Microscopic urinalysis results will be listed only. All laboratory data test results will be included by subject in applicable data listings.

Newly notable laboratory abnormalities (i.e. abnormal post-baseline test results with corresponding normal baseline test results for a given parameter) in clinical chemistry, hematology, and urinalysis will be flagged in corresponding data listings.

The number and percentage of subjects with ALT results greater than upper limit of normal (ULN) and also greater than or equal to 2.5×ULN using both local and central laboratory results will be summarized at each scheduled post-baseline time point and also corresponding to the maximum post-baseline value. If both central and local laboratory results are available for the same visit for a given subject, the highest result will be used for analysis purposes (regardless of the source).

9.4. DTX301 Vector Genome Determination and Viral Shedding

Saliva, stool, and urine samples will be collected at scheduled time points for viral shedding, while blood samples will be collected at scheduled time points for vector genome determination. The presence of DTX301 will be determined by [redacted] A summary table will be presented by sample type and DTX301 dose/prophylactic corticosteroids in the SS. Observed results and change from baseline to each scheduled post-baseline time point will be presented. Observed results will also be presented over time by sample type in a figure for each subject. Relevant individual vector shedding data (baseline value, highest value/timepoint, timepoint where the 3rd consecutive low value after peak) will be provided for each patient and sample type. Median values over time by cohort and sample type will be presented in a figure. Longitudinal displays of individuals data over time by cohort for each sample type will be presented.

Following protocol amendment 5, the assessment of vector genome determination was removed globally and viral shedding sampling was reduced based on medical consideration of results to date and to reduce subject burden.

The planned outputs for vector genome determination will be still created, but patients enrolled under protocol amendment 5 or a subsequent protocol amendment will not be included.

9.5. Vital Sign Measurements

Summary tables will be presented by DTX301 dose/prophylactic corticosteroids for vital sign data, including weight (kg), pulse rate (beats/min), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), respiratory rate (breaths/min), and temperature (C) regardless of body position for subjects in the SS. Observed results and change from baseline to each scheduled post-baseline time point will be presented in a data listing.

9.6. Electrocardiogram Results

An overall ECG interpretation (Normal, Abnormal – Not Clinically Significant, Abnormal – Clinically Significant) will be available based on local reading of ECG data. Shifts from baseline to each scheduled post-baseline time point and 'worst post-baseline value' will be presented to show the number and percentage of subjects in each category by DTX301 dose/prophylactic corticosteroids for subjects in the SS. When considering 'worst post-baseline value', interpretation is ordered as follows: Abnormal – Clinically Significant, Abnormal – Not Clinically Significant, and Normal.

QTc using Bazett's Correction (QTcB) and QTc using Fridericia's Correction (QTcF) parameters will be computed using the following correction methods:

$$\text{Bazett: QTcB (msec)} = \text{QT (msec)} / (\text{RR}/1000)^{1/2}$$
$$\text{Fridericia: QTcF (msec)} = \text{QT (msec)} / (\text{RR}/1000)^{1/3}$$

where RR is reported in milliseconds (msec).

Results for Normal Sinus Rhythm, Ventricular Rate, P-R Interval, QRS Duration, QT Interval, QTcB Interval, and QTcF Interval will be listed only.

9.7. Physical Examination Findings

Complete and targeted physical examination findings will be classified by investigator as Normal, Abnormal – Not Clinically Significant, and Abnormal – Clinically Significant. Shifts from baseline to each post-baseline time point will be presented by body system to show the number and percentage of subjects in each category for subjects in the SS.

10. Statistical Considerations for Data Monitoring Committee Meeting

DMC members will meet after data from Week 12 are available for all subjects in cohort 1, cohort 2 and the first 3 subjects in cohort 3. The decision to proceed to the next dose cohort, expand a dosing cohort, and enroll subjects to cohort 4 (Dosing Process Optimization) will be made after each DMC meeting. The DMC will also hold a meeting at completion of Week 52 for all subjects. Additional DMC meetings may take place, as needed. The full scope of each review will be outlined in the DMC charter.

11. Interim Analysis

An interim analysis may be conducted when week 12 data are available from all subjects from at least 2 dosing cohorts. All tables/listings/figures for the full analysis will be produced for the interim analysis. No interim analysis has taken place.

12. Changes from Analyses Planned in the Protocol

Table 15-1 (Schedule of Events – Outpatient and Inpatient Clinic Visits) in the protocol defines the study day prior to DTX301 dosing as Day 0. However, in accordance with Clinical Data Interchange Standards Consortium (CDISC) conventions, the study day prior to DTX301 dosing will be defined as study day -1.

Potential exploratory analyses of the time-normalized ammonia and/or percent of normal ureagenesis activity by gender has been added.

The DMC will not hold a meeting at completion of Week 12 for the first 3 subjects in cohort 4. It has been agreed to have it only at the completion of Week 52 for all subjects.

No interim analysis has taken place.

[redacted] after patients in cohort 4 have completed the DLT window of 12 weeks or experienced a DLT prior to week 12 completion, in order to provide additional information for Health Canada submission. Patients from cohort 3 and 4 will be pooled together in the N-CRM model.

[redacted] of $AUC_{0\text{-}24h}$ of plasma ammonia and of $AUC_{0\text{-}240min}$ of absolute ^{13}C-urea vs. baseline have been added, along with other additional summary statistics for specific ureagenesis and ammonia parameters.

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14. Appendices

Appendix A: Imputation Algorithm for Partial and Missing Dates

AEs

- If the onset date is completely missing, then the onset date is set to date of administration of study drug.
- If year is present and month and day are missing:
    - If year = year of administration of study drug, then set onset month and day to month and day of administration of study drug
    - If year < year of administration of study drug, then set onset month and day to December 31st.
    - If year > year of administration of study drug, then set onset month and day to January 1st.
- If month and year are present and day is missing:
    - If year=year of administration of study drug and
        - If month = month of administration of study drug then set day to the day of administration of study drug
        - If month < month of administration of study drug then set day to the last day of the month
        - If month > month of administration of study drug then set day to the first day of the month
    - If year < year of administration of study drug then set day to the last day of the month
    - If year > year of administration of study drug then set day to first day of the month
- If the imputed onset date created using the rules above comes after the complete stop date provided, then use the complete stop date

Concomitant Medications

- If start date is completely missing then start date will not be imputed.
- If year is present and month and day are missing then set start month and start day to January 1.
- If start year and start month are present and start day is missing then set start day to the first day of the month.
- If end date is completely missing then end date will not be imputed.
- If year is present and month and day are missing then set end month and end day to December 31.
- If end year and end month are present and end day is missing then set end day to last day of the month.

OTC Deficiency Medical History

- Month and day of diagnosis will be imputed as July 31st, respectively.

- Month and day subject started therapy for OTC deficiency will be imputed as July 31st, respectively.

Absolute ^{13}C-Urea, $AUC_{0\text{-}240min}$ of ^{13}C-Bicarbonate, $AUC_{0\text{-}24h}$ in Plasma Ammonia and urinary Orotic Acid $AUC_{0\text{-}24h}$

- For missing data at individual planned time points:
    - A single missing result between two planned time points that have non-missing results will be imputed by linear interpolation.
    - Two or more adjacent missing results will not be imputed and the $AUC_{t1\text{-}t2}$ measure will be set to missing
    - A single missing result at either the initial or final planned time point will be imputed by linear extrapolation using the first two or last two non-missing results, respectively. In the event that the extrapolated result is negative, the missing result will be set to zero.

Appendix B: Schedule of Events – Outpatient 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
Lafayette grooved pegboard test		X[l]												X[l]
D-KEFS trail making test		X[l]												X[l]
Digital span and arithmetic subtests from WAIS-IV WMI		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; D-KEFS, Delis-Kaplan Executive Function System; 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.**

Appendix C: Schedule of Events – Clinic or Home Visits During the 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.