A Phase 1/2 First-in-human, Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses and Repeat Doses of UX053 in Patients with GSD III
Original Protocol: 20 Jan 2021
Amendment 1: 24 March 2021
Amendment 1.1 (UK-specific): 07 September 2021
Amendment 2: 01 October 2021
Amendment 2.1 (US-specific): 01 October 2021 *(see Table 16)
Amendment 2.2 (UK-specific): 01 October 2021
Amendment 2.3 (US-specific): 03 January 2022
Amendment 3.1 (US-specific): 14 July 2022

Brief Title:	First-in-human Study of UX053 in Patients with GSD III
Product number:	UX053
Indication:	Glycogen Storage Disease Type III (GSD III)
IND Number:	027233
EudraCT Number:	2021-000903-19
Sponsor:	Ultragenyx Pharmaceutical Inc. 60 Leveroni Court Novato, CA 94949 USA
Sponsor's Responsible Medical Monitor:	PPD

This trial will be performed in compliance with the protocol, Good Clinical Practice (GCP), and all applicable regulatory requirements and guidelines.

PROTOCOL AMENDMENT

SUMMARY OF CHANGES AND RATIONALE

UX053-CL101 Amendment 3.1 (US-specific)

14 July 2022

Protocol UX053-CL101 Amendment 2.3 (dated 03 January 2022) has been modified by Amendment 3.1 (US-specific). Amendment 3.1 is considered to be substantial. Minor edits and typographical corrections have also been made. Changes impacting study design and/or conduct are summarized below. In addition to the sections described below where changes occurred, relevant updates were made to the Synopsis.

Section # and Name	Description of Change	Brief Rationale
Section 5.4.4 Potential Benefits; Section 5.5 Risk Minimization; Section 7.1 Study Design; Section 7.1.3 Rationale for Study Design; Section 7.1.4 Dose Rationale; Section 7.2.1 Duration of Subject Participation; Section 8.1 Inclusion Criteria; Section 8.2 Exclusion Criteria; Section 8.5 Screening Requirements; Section 9 Interventions; Section 9.1.2 Preparation and Administration; Section 9.3 Nutrition Management; Section 9.7 Treatment Compliance; Section 9.8.1 Treatment Assignment; Section 9.8.2; nearly all subsections of Section 10 Assessments; Section 11.1 Sample Size Determination; Section 11.2 Analysis Sets; Section 11.3.1 General Principles; Section 11.6 Anti-drug Antibody Analyses; Section 11.7 Efficacy Analyses; Section 11.11 Planned Analyses; Figure 1 UX053-CL101 Study Design; Schedule of Events Table 4, Table 5, and Table 6; Schedule of PK Assessments Table 8; Appendix 2	Subjects who complete the 90-day safety follow-up in the Single Ascending Dose (SAD) cohorts can elect to enter into open-label repeat dose (OL-RD) cohorts to receive an additional 4 doses of open-label UX053 administered every 4 weeks (Q4W). These subjects will be rescreened for participation in the OL-RD cohorts prior to redosing; eligibility can be combined with assessments at the SAD Day 90 Visit. During rescreening for OL-RD cohorts, subjects will be assessed for status of diet optimization and stability based on nutrition data collected during participation in SAD cohorts and rescreening for OL-RD cohorts. Reflecting this change, there are now 2 sets of RD cohorts: the previously included randomized, double-blind, placebo-controlled RD (DB-RD) cohorts and the newly added OL-RD cohorts. Initiation of OL-RD cohorts runs in parallel with initiation of DB-RD cohorts, guided by Data Monitoring Committee (DMC) review. The timing of the DMC reviews was updated to reflect this. A new schedule of events table was added to indicate the timing of assessments for the OL-RD cohorts and the Schedule of pharmacokinetic (PK) Assessments Table 8 was updated to reflect the addition of OL-RD cohorts treated Q4W.	The optimal dose interval for UX053 is not known. Allowing subjects who participated in SAD cohorts to enroll in OL-RD cohorts will allow for the investigation of safety, PK, and pharmacodynamic (PD) effects of repeat dosing of UX053 with a Q4W dosing regimen. The Q2W regimen is expected to be the maximum frequency of dosing based on nonclinical studies, and a less frequent dosing regimen may be possible based on PK data in humans.
Section 10.5.1 Controlled Fasting Challenge	Section 10.5.1 was added to reflect the addition of a controlled fasting challenge (CFC) as an optional assessment for subjects in RD cohorts.	To assess if CFC is useful to identify a clinically significant improvement in glycemic status with UX053 treatment.
Section 7.1 Study Design	A schematic illustrating nutrition optimization and stabilization requirements during screening for the DB-RD cohorts was added.	To clarify the nutrition optimization and stabilization requirements by cohort.

Section # and Name	Description of Change	Brief Rationale
Section 8.1 Inclusion Criteria; Section 8.2 Exclusion Criteria; Section 9.6 Restricted or Prohibited Medications, Devices, and Procedures	One new inclusion criterion and 1 new exclusion criterion were added to allow rescreening of subjects from the SAD cohorts as they enter the OL-RD cohorts (noted above). Existing eligibility criteria that referred to RD cohorts were edited to specify how the criteria apply to the new OL-RD cohorts. The newly added inclusion criterion is: For subjects rescreening into OL-RD cohorts, after treatment with UX053 in a SAD cohort, subjects must meet the following criteria: a. If a significant rise in ALT occurs after the prior dose, ALT should show a decreasing trend toward the subject's baseline value b. Total bilirubin is within normal limits c. Platelets are within normal limits d. INR is within normal limits The additional exclusion criterion is: For subjects rescreening into OL-RD cohorts, any of the following after treatment with UX053 in a SAD cohort [are excluded]: e. New or worsening symptoms of liver disease (including new or worsening hepatomegaly) along with any increase in transaminase levels f. Receipt of any blood product administration (eg, packed red blood cells, platelet, fresh frozen plasma) for management of consumptive coagulopathy g. An ALT level that is ≥ 8x ULN and > 2x the subject's baseline value in the absence of an alternative explanation	Subjects who receive a single dose of UX053 are no longer treatment naïve. Subject-level dose interruption criteria were adapted for the purpose of redosing these subjects in the OL-RD cohorts.

Section # and Name	Description of Change	Brief Rationale
Section 7.1 Study Design	All SAD cohorts will consist of at least 2 subjects. Previously, Cohorts 1S and 2S had 2 subjects each and Cohort 3S and Cohort 4S had 3 subjects each. The text indicating that subjects will be added in blocks of 4 was removed.	Safety data from at least 2 subjects are required for the decision to proceed with the next cohort(s). If further characterization of safety, PK, and PD at a given dose level is desired, individual subjects may be added to SAD cohorts as needed.
Section 5.4.1.1 Potential Risks Identified from Nonclinical Studies; Section 7.1 Study Design; Section 7.1.4 Dose Rationale; Section 9 Interventions	Dose escalation plans were clarified. Ultragenyx aims to test at least 3 dose levels of UX053. Dose levels for each cohort may be altered depending on safety, PK, and PD findings from prior cohorts. If needed to evaluate safety and dose-response relationship additional cohorts may be added to test doses up to 0.30 mg/kg. Cohorts 4S and 4R, in which subjects are to receive single or multiple doses of UX053 at 0.30 mg/kg, respectively, are now optional.	All cohorts beyond Cohort 1S are contingent on supportive safety, PK, and PD data from prior cohorts. Based on preliminary data from Cohort 1S and nonclinical PK/PD modeling, it is unlikely that doses as high as 0.30 mg/kg will be needed for maximum biological effect.
Section 7.1.2 Number of Subjects; Section 11.1 Sample Size Determination	The total number of subjects included in the study was updated to approximately 18, reflecting 6 planned subjects in Cohorts 1S, 2S, and 3S and 12 planned subjects in Cohorts DB-1R, DB-2R, and DB-3R. Additional subjects may be added to SAD or RD cohorts for further characterization of safety.	The updated total number of subjects reflects the changes in study design.
Section 7.1 Study Design	Reference to the Pharmacy Manual regarding guidance on dose reductions was removed. If a subject in a RD cohort (either DB or OL) develops a treatment-emergent adverse event (TEAE)/serious TEAE ≥ Grade 3 that is considered by the Investigator to be related to study drug or an intolerable TEAE in a RD cohort, any further study drug administration and any subject-level dose reductions should be discussed with the Medical Monitor.	Investigators should not redose subjects who have experienced UX053-related TEAEs until after discussion with the Medical Monitor.

Section # and Name	Description of Change	Brief Rationale
Section 7.1 Study Design; Schedule of Events Table 4 and Table 6	Text was edited to clarify that telephone calls regarding the use of continuous glucose monitoring (CGM) and handheld glucometer (HHG) devices will occur as needed, outside of scheduled study visits.	To clarify the timing of telephone calls related to CGM and HHG data collection.
Section 7.1 Study Design	Text was added to clarify that subjects within a given RD cohort can be dosed simultaneously. After initiation of repeat dosing, subjects will follow a Q2W (DB-RD cohorts) or Q4W (OL-RD cohorts) dosing regimen.	To clarify the interval between dosing subjects within RD cohorts.
Section 8.1 Inclusion Criteria	The italicized text was added to the following inclusion criterion "Willing and able to provide access to medical records surrounding medical treatment that occurred prior to enrollment *and during the study*."	To ensure complete capture of relevant safety data.
Section 8.2 Exclusion Criteria; Section 9.6 Restricted or Prohibited Medications, Devices, and Procedures	The exclusion criterion "Planned surgery, including dental surgeries, during the SAD Period for subjects in Part 1 or prior to Week 10 of the RD Period for subjects in Part 2" was updated to "Planned surgery, including dental surgeries, during the study." This text was also updated in the section regarding restricted procedures.	To simplify for greater clarity.
Section 10.4.4.4 Nutrition Diary; Schedule of Events Table 4 and Table 6	For all cohorts, nutrition diary recordings will now be recorded at least 3 consecutive days per week through Screening and for the 3 consecutive days in the week leading up to each subsequent visit.	To reduce subject burden.
Schedule of Events Table 6	Assessments for serum CK-M/B, troponin I, and CK, and plasma BNP were added at Weeks 2, 4, 6, and 8 for the DB-RD cohorts. The assessment for plasma BNP was removed from ISV.	ToAssessments were added to facilitate evaluation of potential PD effects on heart and skeletal muscle. The assessment for plasma BNP is no longer performed at ISV, but can be performed during screening (Nutrition Optimization Visits [NOVs] and Stabilization Visit [STV]) at the discretion of the Investigator.
Schedule of Events Table 4 and Table 6; Appendix 3	Assessments for urine beta hydroxybutyrate and whole blood for RNA analysis were removed for all cohorts.	To reduce subject burden.

Section # and Name	Description of Change	Brief Rationale
Schedule of Events Table 6	Assessments for urine beta hydroxybutyrateThe physical exam and weight assessment was added to Weeks 3, 5removed from Week 12, 24, and 736 for the DB-RD cohorts.	To facilitate evaluation of potential PD effects on ketosis.To reduce subject burden.
Schedule of Events Table 4	The Day 42 Visit was removed for the SAD cohort.	To reduce subject burden.
Schedule of Events Table 6	Assessment of serum ketones was removed at Week 9 for the DB-RD cohorts. Serum ketones was also changed to capillary ketones for DB-RD cohorts. Also for the DB-RD cohorts, ketones are no longer assessed at the NOV (previously optional per discretion of the Investigator) and Weeks 1, 12, 24, and 36.	Assessment of serum ketones is not required at WeekNOV, and Weeks 1, 9, 12, 24, and 36 for the DB-RD cohorts. The assessment of capillary ketones instead of serum ketones for RD cohorts aligns with the addition of an optional CFC for RD cohorts.
Schedule of Events Table 6	The schedule of events tables for the RD cohorts reflects that the imaging assessments (liver magnetic resonance imaging [MRI], magnetic resonance spectroscopy [MRS], FibroScan or ultrasound elastography) can occur throughout rescreening (OL-RD) or screening (DB-RD). Imaging assessments were also removed for the DB-RD EOS III W48/ET Visit.	To reduce subject burden.
Section 8.3.2 Subject-level Redosing and Stopping Criteria; Section 9.1.1 Premedications; Section 9.1.2 Preparation and Administration; Section 9.1.3 Clinical Observation and Supportive Care; Section 9.5 Rescue Medications; Section 11.4 Safety Analyses	Infusion-associated reaction (IAR) was changed to infusion-related reaction (IRR) throughout the protocol.	The use of IRR better aligns with Medical Dictionary for Regulatory Activities (MedDRA) terminology.
Schedule of Events Table 6	Prothrombin time/international normalized ratio (PT/INR) will be assessed at all scheduled study visits in the Treatment and Follow-up Period for the DB-RD cohorts.	To facilitate early detection of consumptive coagulopathy and liver injury, both are a potential risk of UX053.

Section # and Name	Description of Change	Brief Rationale
Schedule of Events Table 6; Section 10.5.5; Appendix 3	Assessments for plasma glucose tetrasaccharide (Glc_4) were removed from the study and the heading title for Section 10.5.5 was changed from "Limit Dextrins" to "Glucose Tetrasaccharide." Assessments for urine Glc_4 were added at Weeks 3, 4, 5, 6, 7, and 9 for DB-RD cohorts.	Plasma Glc_4 is no longer required, and removal of this assessment will reduce subject burden. The timing of urine Glc_4 was changed to improve temporal resolution of this PD biomarker.
Schedule of Events Table 6 Section 10.5.7 Exploratory Future Use Blood Cell Pellet, Serum, and Plasma Collection.	The row titled "Blood Cell Pellet for Future Use" in all schedule of events tables was removed, and the row Serum & Plasma for Future use" was updated to include white blood cells (WBC) and red blood cells (RBC). Similar language changes were made in Section 10.5.7.	To align language between region-specific protocols. There is no operational change as a result of this language change.
Table 7 Schedule of Vital Sign Assessments	Table 7 Schedule of Vital Sign Assessments across SAD and RD cohorts was consolidated and simplified to reflect consistency in vital sign assessments.	The schedule of vital sign assessments on infusion visits is the same for all infusions, regardless of cohort.
Section 8.3.2 Subject-level Redosing and Stopping Criteria	"Safety labs will be obtained at Weeks 1, 3, 5, 7, and 9 of the RD Period, corresponding to 1 week after each dose of study drug" was removed from Section 8.3.2 Subject-level Redosing and Stopping Criteria.	This information is not appropriate for this section.

Section # and Name	Description of Change	Brief Rationale
Section 5.4.2 Potential Risks of Premedication; Section 5.5 Risk Minimization; Section 7.1.4 Dose Rationale; Section 8.3.2 Subject-level Redosing and Stopping Criteria; Section 9.1.1 Premedications; Section 9.5 Rescue Medications	Text was added throughout relevant sections to reiterate guidance from Table 11, noting that epinephrine should be administered if a subject develops an IRR that meets diagnostic criteria for anaphylaxis. For all other hypersensitivity reactions ≥ Grade 2 in severity suspected to be due to immune activation, dexamethasone should e the initial rescue medication. Text was added to Section 9.5 to account for rescue medications in the event of severe consumptive coagulopathy and profound complement activation. Also in this section, text was updated to clarify that premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications. As previously noted in , the following text was added to Section and Section : "Additional treatment, including a complement inhibitor (such as eculizumab) may be considered."	To provide additional clarity on the use of rescue medications in the event of potential risks of UX053 treatment.
Section 11.2 Analyses Sets	The terminology regarding the analyses sets was updated. The Safety Analysis Set will include all enrolled subjects, defined as subjects deemed eligible for treatment at D0 in the SAD cohorts, and those randomized in the DB-RD cohorts, and those who continue into OL-RD cohorts from SAD cohorts. A Per Protocol (PP) Analysis Set, consisting of subjects who complete the study without major protocol deviations that could compromise PD or efficacy assessments may be analyzed as described in the Statistical Analysis Plan (SAP). The PK Analysis Set is a subset of subjects in the Safety Analysis Set who have at least 1 evaluable investigational product (IP) concentration. The PK Analysis Set will be used for the analysis of PK endpoints.	Changes were made to align with the SAP.

Section # and Name	Description of Change	Brief Rationale
Schedule of Events Table 4	Footnote a in the SAD cohort's schedule of events table was updated to indicate that there must be a minimum of 10 days between any assessments that are measured at both the Screening and the Baseline Visit.	To provide clarification regarding the timing required between assessments taken at the Screening and Baseline Visit for SAD cohorts.
Section 10.4.1.1 Liver Magnetic Resonance Imaging; Section 10.4.1.2 Liver Magnetic Resonance Spectroscopy	Study Reference Manual was changed to Study Imaging Manual.	Instructions related to liver MRI and MRS are provided in the Study Imaging Manual.
Schedule of Events Table 4 and Table 6; Section 10.4.1.1 Liver Magnetic Resonance Imaging	If feasible, quantitation of glycogen using nuclear overhauser enhancement (glycoNOE) (Zhou et al., 2020) of the liver, may be collected at the time of the liver MRI/MRS.	GlycoNOE may provide additional insight into changes in glycogen content in the liver.
Section 8.1 Inclusion Criteria	The word "fertile" was removed from the following entry criterion "Females of childbearing potential must have a negative pregnancy test at screening (and rescreening, as applicable) and be willing to have additional pregnancy tests during the study. Subjects of childbearing potential or fertile males who are sexually active with partners of childbearing potential must consent to use a highly effective contraceptive method, as described in Appendix 4, from the Period following the signing of the informed consent through 30 days after last dose of study drug."	Assessment of fertility for males in this study is not conducted.
Section 5.5 Risk Minimization; Section 7.1.4 Dose Rationale; Section 9.1.2 Preparation and Administration; Schedule of Events Table 8	As noted in the prior amendment, the study drug infusion rate schedule may require modification, including lengthening of total infusion time, to minimize the risk of IRRs in individual subjects. Text regarding slowing the infusion rate was edited and added in Section 7.1.4 and Section 9.1.2. Reflecting this adaptive design, the following text was added as a footnote to Table 8: "If the total infusion time is extended beyond 4 hours, a blood sample should be collected at the new end of infusion (EOI) time. If the new EOI time overlaps with a prespecified time point, sampling should continue at the next scheduled time point"	The rate of infusion of study drug may be adjusted to minimize the risk of IRRs.

Section # and Name	Description of Change	Brief Rationale
Section 7.1 Study Design	Text was added defining a stable diet as remaining within the nutritional guidelines for adults with GSD III based on expert recommendations.	To clarify the definition of a stable diet.
Section 7.1 Study Design	The following text was removed "Mean weekly protein intake < 20% of total calorie intake and mean weekly carbohydrate intake ≥ 60% of total calorie intake for subjects will be considered protocol deviations, notwithstanding temporary changes to diet due to illness. The classification of such deviations will be determined by the magnitude, frequency, and duration of excursions beyond the specified guidelines."	There is no need to call out specific examples of protocol deviations, and a definition of stable diet was added separately.
Section 10.4.4.3 GNE Myopathy Functional Activities Scale Expanded Version	The GNE Myopathy Functional Activities Scale (GNEM-FAS) will no longer be scored by the clinician. The clinician will administer the assessment and provide the raw values for Ultragenyx to score.	To reflect the change in scoring of the GNEM-FAS.
Section 7.1 Study Design	Text was added indicating that a future amendment may occur to add an open-label extension after an interim review of safety, PK, and PD data.	This text was added to reflect potential future changes to the protocol.
Section 8.2 Exclusion Criterion	CCI [redacted]	CCI [redacted]
Section 8.2 Exclusion Criterion	In the following sentence within exclusion criterion 10, "asymptomatic cardiomyopathy" was updated to "mild cardiomyopathy:" "Asymptomatic cardiomyopathy and left ventricular hypertrophy (LVH) are allowed.	New York Heart Association (NYHA) Function Capacity II and below are allowed, not just asymptomatic cardiomyopathy.
Title Page	A brief study title was added to the title page. The study title was also edited.	Updated per recent regulatory guidance. and to reflect changes in this amendment (ie, addition of an open-label cohort).

Section # and Name	Description of Change	Brief Rationale
Section 6 Objectives and Endpoints	Reflecting the addition of a Q4W dosing regimen, the secondary endpoint was updated to "PK parameters of *AGL* mRNA and ATX95, which may include T_{max}, C_{max}, AUC_{last}, AUC_{inf}, AUC_{tau} (RD cohorts only), R_{AUC} (RD cohorts only), T_{last}, $T_{1/2}$, CL, V_{ss}"	The secondary endpoint was updated to reflect that both Q2W and Q4W dosing regimens will be evaluated.
Section 5.4.2 Potential Risks of Premedication; Section 7.1 Study Design; Section 7.1.4 Dose Rationale; Section 8.3.2 Subject-level Redosing and Stopping Criteria; Section 9.1.1 Premedications; Section 9.1.3 Clinical Observation and Supportive Care; Section 9.5 Rescue Medications	The sentence "If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for all subjects' subsequent infusions" was updated to "If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor." Similar edits were made in sections that referenced this guidance.	Subjects may have different sensitivities, requiring an individualized assessment for the use of additional premedication.
Schedule of Events Table 4 and Table 6; Section 10.4.3.1 Creatine Kinase and Myoglobin Section 10.4.3.2; Calf Magnetic Resonance Spectroscopy; Section 10.5.3 Serum Biotinidase; Appendix 3	Serum myoglobin, serum biotinidase, and calf MRS were removed from the study for all cohorts.	To reduce subject burden, assessments for these exploratory PD endpoints were removed.
Schedule of Events Table 6	The Week 12, 24, and 36 Visits for DB-RD cohorts were changed to be conducted at home. As a result, Fibroscan or ultrasound elastography will be conducted at Week 10 instead of Week 12; and GNEM-FAS, Patient-reported Outcomes Measurement Information System (PROMIS), and handheld dynamometry (HHD) will be assessed at Week 10, and not at Weeks 12, 24, and 36.	To reduce subject burden.
Appendix 5	Text regarding Data Protection, Anonymization, and Security in Appendix 5, was updated to reflect the latest regulatory guidance.	Updated per regulatory guidance and for clarity.

Section # and Name	Description of Change	Brief Rationale
Section 5.5 Risk Minimization	Text regarding the collection of safety labs in Section 5.5 Risk Minimization was updated to align with the schedule of events.	To correct and align protocol text with the schedule of events.

2. SYNOPSIS

Sponsor: Ultragenyx Pharmaceutical Inc. (Ultragenyx)

Ultragenyx Product: UX053

Title: A Phase 1/2 First-in-human, Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses and Repeat Doses of UX053 in Patients with GSD III

Development Phase: Phase 1/2

Introduction

UX053 is an mRNA-based biologic that is being developed for the treatment of Glycogen Storage Disease Type III (GSD III). The purpose of this first-in-human (FIH) trial is to investigate the safety, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary efficacy of UX053 in adults with GSD III.

Objectives and Endpoints

Table 1: Primary and Secondary Endpoints

OBJECTIVES	ENDPOINTS
Primary	
Evaluate the safety of UX053 in adults with GSD III	The incidence and severity of TEAEs, serious TEAEs, and related TEAEs in the SAD and RD cohorts
Secondary	
Characterize the PK of UX053 in adults with GSD III	PK parameters of *AGL* mRNA and ATX95, which may include T_{max}, C_{max}, AUC_{last}, AUC_{inf}, AUC_{tau} (RD cohorts only), R_{AUC} (RD cohorts only), T_{last}, $T_{1/2}$, CL, V_{ss}

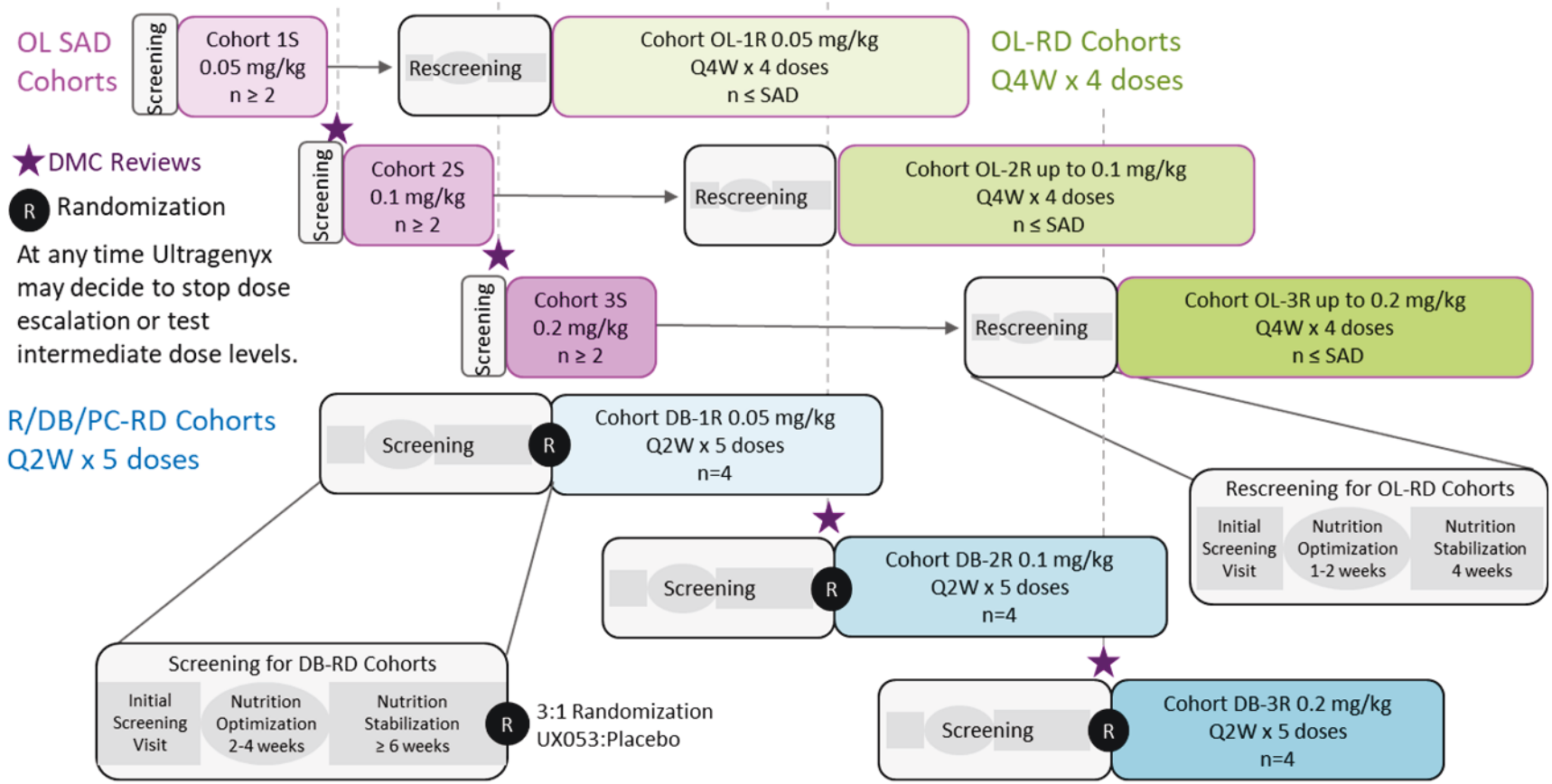
AGL, amylo-α-1,6-glucosidase 4-alpha-glucanotransferase; AUC, area under the concentration-time curve; AUC_{inf}, AUC from time 0 to infinity; AUC_{last}, AUC from time 0 to the last measurable concentration; AUC_{tau}, AUC from time 0 to end of dosing period; CL, clearance; C_{max}, maximum observed concentration; PK, pharmacokinetics; R_{AUC}, accumulation ratio (calculated as AUC after repeat dose / AUC after a single dose); RD, Repeat Dose; SAD, Single Ascending Dose; $T_{1/2}$, half life; TEAE, treatment-emergent adverse events; T_{last}, time of last measurable concentration; T_{max}, time of maximum observed concentration; V_{ss}, volume of distribution at steady state.

Tertiary CCI [redacted] and exploratory objectives CCI [redacted] and their corresponding endpoints are provided in the Statistical Analysis Plan (SAP).

Study Design

UX053-CL101 is a phase 1/2 FIH study to evaluate the safety, tolerability, and PK of single ascending dose (SAD) and repeat doses (RD) of UX053 in patients with GSD III (Figure 1). The SAD cohorts will be open label (OL). There will be 2 types of RD cohorts: 1) OL-RD and 2) randomized, double-blind (DB), and placebo-controlled RD (referred to as DB-RD). Treatment-naïve subjects enter the SAD and DB-RD cohorts. Subjects in the SAD cohorts who complete the SAD 90-day Follow-up Period can enter OL-RD cohorts. Details for each of these cohorts are described below.

Figure 1: UX053-CL101 Study Design



Note: Additional subjects may be included in any cohort at the discretion of Ultragenyx. Dose levels for any cohort may be reduced depending on safety findings from prior cohorts. Additional cohort(s) at dose levels between 0.05 mg/kg to 0.3 mg/kg may also be included pending review of safety data.

Note: Dose escalation is guided by review of ≥ 2 and ≥ 6 weeks of safety data for the SAD and RD cohorts, respectively. Higher dose cohorts may not be initiated, depending on safety and PD findings from prior cohorts, as determined by Ultragenyx (with input from the DMC for the RD cohorts.

DB, randomized double-blind placebo-controlled; OL, open-label; Q2W, every 2 weeks; Q4W, every 4 weeks; RD, Repeat Dose; SAD, Single Ascending Dose

Single Ascending Dose Cohorts

The SAD cohorts evaluate safety and PK of single doses of UX053 in treatment-naïve subjects. All subjects in the SAD cohorts will undergo a Screening Visit prior to their Baseline Visit. There are 3 planned SAD cohorts, each consisting of at least 2 subjects, all of whom will receive open-label UX053 (Figure 1). The planned dose escalation will proceed from 0.05 mg/kg in Cohort 1S, to 0.10 mg/kg in Cohort 2S, and to 0.20 mg/kg in Cohort 3S. An independent Data Monitoring Committee (DMC) will review at least 2 weeks of safety data for at least 2 subjects within a SAD cohort and the cumulative data from all subjects in prior dosing cohorts (when applicable) before deciding to proceed with the next SAD cohort.

Each subject in the SAD cohorts will be followed for a Follow-up Period of 90 days after dosing. At the SAD Day 90 Visit, marking completion of the Follow-up Period, subjects can be rescreened for entry into the OL-RD cohorts described below.

Open-Label Repeat Dosing Cohorts (Extension for SAD Cohorts)

The OL-RD cohorts serve as a short-term extension for subjects who participated in a SAD cohort. The OL-RD cohorts evaluate the safety and PK of 4 additional OL doses of UX053 in subjects who received a single dose of UX053 in a SAD cohort and completed the 90-day Follow-up Period. Subjects from the SAD cohorts are rescreened for participation in the OL-RD cohorts; eligibility for additional dosing in the OL-RD cohorts can be combined with assessments at the SAD Day 90 Visit.

During rescreening, a nutrition assessment will be completed at an Initial Screening Visit (ISV) to determine if the subject's diet meets the nutritional guidelines for adults with GSD III based on expert recommendations (Table 2). OL-RD subjects will proceed through rescreening as shown in Figure 2 based on whether they meet these nutritional guidelines.

Table 2: Nutrition Guidelines for Adults with GSD III

High protein intake with a target of ≥ 25% of total calories
Low complex carbohydrates intake with a target of < 50% of total calories
Minimal intake of simple sugars
Avoidance of fasting
Consideration of high-protein bedtime snack or a high-protein formula for overnight enteral feeding

Based on expert guidelines (Kishnani et al., 2010)

The first day of the ISV (which may coincide with the SAD Day 90 Visit) is followed by 3 consecutive days of nutrition diary collection. If a subject's diet during participation in a SAD cohort meets nutritional guidelines, and continues to do so at the ISV, the subject can proceed directly to the OL-RD Nutrition Stabilization Visit (STV) as soon as the ISV assessments are completed.

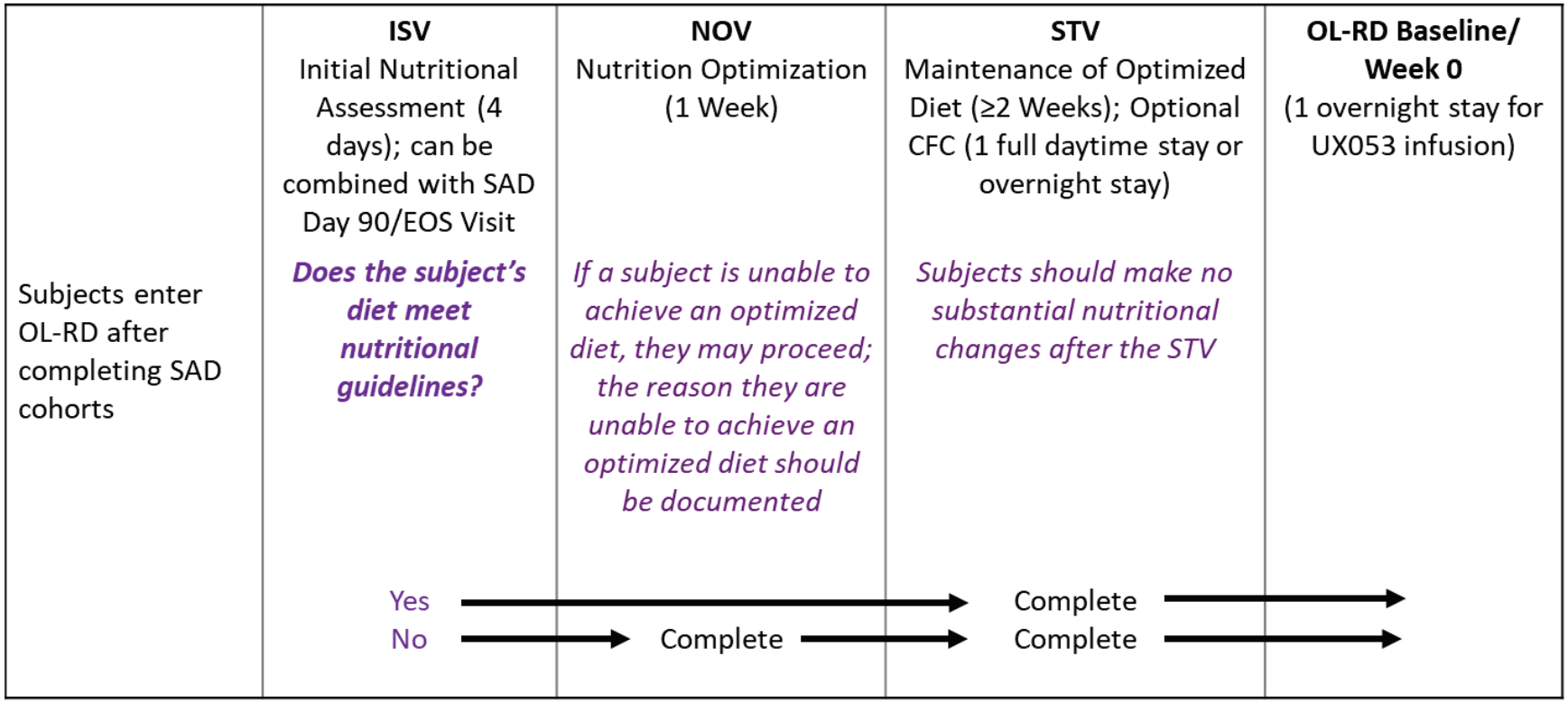
If the subject's diet does not meet nutritional guidelines, a Nutrition Optimization Visit (NOV) is performed. At this visit, nutritional counseling is provided to the subject on dietary changes needed to comply with GSD III-specific nutrition guidelines (Table 2). Nutrition diary and continuous glucose monitor (CGM) data will be reviewed by the Investigator and safety

laboratory tests may be obtained at the discretion of the Investigator. After 1 week of nutrition optimization (approximately 2 weeks after the ISV), the subject proceeds to the STV (occurring approximately 2 weeks before the subject's OL-RD Baseline/Week 0 Visit) and thereafter maintains a stable diet as defined by the nutritional guidelines for GSD III (Table 2) for the remainder of the study. If an OL-RD subject is unable to achieve an optimized diet, they are still eligible to proceed to the STV. The reason for the inability to achieve an optimized diet should be documented and the subject should make no substantial nutrition changes for the remainder of the study.

For selected sites and subjects, the OL-RD STV includes an optional controlled fasting challenge (CFC). Prior to the CFC, a morning cortisol is obtained between 6 and 9 am. The CFC is conducted during the subject's stay in the hospital, usually overnight, but can be conducted during the day. For the same sites and subjects, another optional CFC occurs at the Week 16 Visit; only subjects who completed the optional CFC at STV complete the CFC at Week 16. Additional details regarding the CFC are in Section 10.5.1.

Beginning with the OL-RD Baseline/Week 0 Visit, subjects in the OL-RD cohorts will receive 4 additional OL doses of UX053 Q4W at the same or lower dose than they received in the SAD cohorts, based on ongoing assessment of safety data.

Figure 2: Rescreening Schematic for OL-RD Cohorts



CFC, controlled fasting challenge; EOS, End of Study; ISV, Initial Screening Visit; NOV, Nutrition Optimization Visit(s); OL, open-label; RD, repeat dose; SAD, single ascending dose; STV, Stabilization Visit.

Randomized, Double-Blind, and Placebo Controlled Cohorts

The DB-RD cohorts will evaluate the safety and PK of 5 doses of UX053 or placebo in treatment-naïve subjects. All DB-RD subjects will participate in a Screening Period (approximately 8 to 12 weeks) (Figure 3), in which subjects will be screened for study eligibility and assessed for the need to optimize their diet based on nutrition guidelines for adults with GSD III based on expert recommendations (Table 2). Within the first 2 weeks after the ISV, the Investigator will review nutrition diary data to determine if nutrition optimization is needed. If nutrition optimization is not needed, the STV occurs in clinic approximately 2 weeks after the ISV, and the Baseline/Week 0 Visit occurs approximately 4 weeks after the STV to demonstrate 6 weeks of nutrition stability.

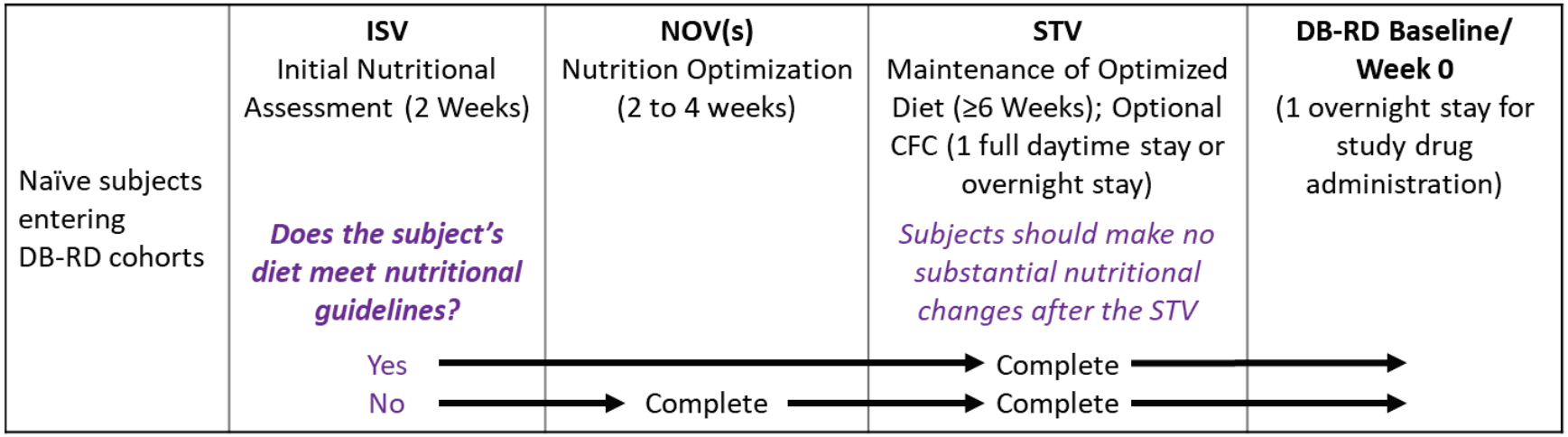
If nutrition changes are needed, a 2- to 4-week period of nutrition optimization will be performed to comply with GSD III-specific nutrition guidelines (Table 2). In this situation, the first NOV occurs as a phone visit approximately 2 weeks after the ISV. During nutrition optimization, NOV phone visits occur weekly or more frequently as needed; nutrition diary and CGM data are reviewed; and safety laboratory tests may be obtained at the discretion of the Investigator. Nutritional counseling is provided to the subject. When the Investigator determines that the subject has achieved nutritional optimization the STV is performed.

The Baseline/Week 0 Visit occurs as least 6 weeks after the STV. Due to the staggered nature of the cohorts (described below), a subject may be in nutrition stabilization for longer than 6 weeks before their Baseline Visit; in such instances, the total duration of the Screening Period may exceed 12 weeks. Nutrition guidance and monitoring for this study was established with available health authority guidance, and greater detail is available in the Study Reference Manual.

After the STV, no substantial nutrition changes should be made through the remainder of the study, notwithstanding temporary changes due to illness.

For selected sites and subjects, the DB-RD STV includes an optional controlled fasting challenge (CFC). Prior to the CFC, a morning cortisol is obtained between 6 and 9 am. The CFC is conducted during the subject's stay in the hospital, usually overnight, but can be conducted during the day. For the same sites and subjects, another optional CFC occurs at the Week 10 Visit; only subjects who completed the optional CFC at STV complete the CFC at Week 10. Additional details regarding the CFC are in Section 10.5.1.

Figure 3: Screening Schematic for the DB-RD Cohorts



CFC, controlled fasting challenge; DB, double blind; ISV, Initial Screening Visit; NOV, Nutrition Optimization Visit(s); RD, repeat dose; STV, Stabilization Visit.

In DB-RD cohorts, treatment-naive subjects will be randomized 3:1 to UX053 or placebo in ascending dose cohorts (4 subjects per cohort) (Figure 1). DB-RD subjects will receive study drug every 2 weeks (Q2W); a total of 5 doses of study drug are planned. The planned dose levels are 0.05 mg/kg in cohort DB-1R, 0.10 mg/kg in cohort DB-2R, and 0.20 mg/kg in cohort DB-3R.

Initiation of RD Cohorts (Both OL-RD and DB-RD)

Initiation of RD cohorts is as follows:

- Initiation of Cohort DB-1R and OL-1R will be guided by DMC review of at least 2 weeks of safety data from at least 2 Cohort 2S subjects and any available data from all subjects in prior and ongoing cohorts.
- Initiation of Cohort OL-2R and DB-2R will be guided by DMC review of either 1) at least 6 weeks of safety data from at least 4 subjects within Cohort DB-1R or 2) at least 12 weeks of safety data from at least 3 subjects within Cohort OL-1R (whichever is available first). In both of these scenarios, all available data from all subjects in prior and ongoing cohorts will also be reviewed.
- Initiation of Cohort OL-3R and Cohort DB-3R will be guided by DMC review of either 1) at least 6 weeks of safety data from at least 4 subjects within Cohort DB-2R or 2) at least 12 weeks of safety data from at least 3 subjects within Cohort OL-2R (whichever is available first). In both of these scenarios, all available data from all subjects in prior and ongoing cohorts will also be reviewed.

Cohort- and Subject-level Dose Selection and Modification for All Cohorts (SAD, OL-RD, and DB-RD)

At any time Ultragenyx may decide not to initiate a planned dose cohort. Dose levels for each cohort may be altered depending on safety, PK, and/or PD findings from prior cohorts. If needed to evaluate safety and dose-response relationships, additional cohorts may be added to test doses up to 0.30 mg/kg or additional subjects may be added to a cohort at the discretion of Ultragenyx to further characterize safety, PK, and PD effects, with input from the DMC. In every case, the safety of a single dose will be evaluated prior to repeat doses at the same dose level.

If a subject in an RD cohort develops a treatment-emergent adverse event (TEAE)/serious TEAE ≥ Grade 3 that is considered by the Investigator to be related to study drug or an intolerable TEAE, subject-level dose reductions are allowed in consultation with the Medical Monitor. Ultragenyx will notify the DMC immediately if any subject experiences an event that satisfies subject-level stopping criteria (Section 8.3.2) or study-level stopping criteria (Section 8.3.3).

Glucose and Nutrition Data Collection by Study Subjects

At the Screening Visit for the SAD cohorts and ISV for the DB-RD cohorts, all subjects will receive CGM and handheld glucometer (HHG) devices and will be trained on the proper use of these devices. Subjects will also be trained on completion of the nutrition diary during screening. Subjects entering the OL-RD cohorts do not need to be retrained if compliance was acceptable during participation in the SAD cohorts. When CGM data is being collected, all subjects will connect their CGM and HHG devices to a study-provided laptop weekly and upload CGM and HHG data. At remote visits, in conjunction with assessments performed by a home health nurse,

the site will contact subjects by telephone or videoconference to confirm the CGM device is properly in use and data was uploaded; answer any questions regarding or help troubleshoot (if necessary) CGM device use; and review CGM data, HHG data (when applicable), and nutrition diary entries; outside of scheduled visits, these telephone calls will occur as needed. At in-clinic visits, a conversation between the site and the subject should occur to ensure CGM compliance and troubleshoot if needed.

Drug Administration, Dose Interval, and Blinding

UX053 will be administered as an intravenous (IV) infusion over the course of at least 4 hours, with a slower rate of infusion for the first hour to minimize the risk of hypersensitivity or anaphylactoid-type reactions (refer to the Pharmacy Manual for additional infusion details) (Table 3 and Section 9). The infusion rate may be slowed to minimize the risk of infusion-related reactions (IRRs) in individual subjects.

Premedication and Rescue Medication

All subjects, including those treated with placebo, will receive premedication at least 1 hour prior to the infusion, consisting of oral paracetamol/acetaminophen (500 mg) or ibuprofen (400 to 800 mg), an H2 blocker (eg, famotidine 20 mg or equivalent dose of another H2 blocker), and an H1 blocker (eg, cetirizine 10 mg or equivalent dose of another H1 blocker). For the premedication, paracetamol/acetaminophen is preferred over ibuprofen.

At the discretion of the Investigator, with input from Ultragenyx and the DMC, and based on the emerging safety profile of UX053, additional premedications and rescue medications may be used to reduce the risk and severity of immune reactions in RD cohorts. Premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications in patients with GSD III.

If a subject develops an IRR that meets diagnostic criteria for anaphylaxis (Section 8.3.3), epinephrine should be administered as initial rescue medication. For all other hypersensitivity reactions ≥ Grade 2 in severity suspected to be due to immune activation, dexamethasone should be the initial rescue medication. The Medical Monitor must be notified if any rescue medication is used.

If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor.

Section 8.3.2 provides subject-level redosing criteria, stopping criteria, and additional instructions on how to proceed in the event of an IRR.

Subjects will have cardiorespiratory monitoring throughout the infusions and will stay overnight for visits in which they receive study drug and be observed in an inpatient or observational unit for 24 hours after the end of each infusion. Prior to discharge, all the following criteria must be met:

- All scheduled study assessments have been completed.
- No evidence of vital sign instability or clinical instability in the opinion of the investigator.
- Any signs or symptoms of potential IRRs are resolving or resolved.

Guidance on signs and symptoms that subjects should be vigilant for upon discharge and how to proceed in the event that these signs and symptoms manifest are provided in Section 9.1.4.

Table 3: Study Investigational Product, Premedication, and Rescue Medication

	Intervention[a]					
	UX053	**Placebo**	**Paracetamol/ acetaminophen or ibuprofen[b]**	**H1 blocker; cetirizine or equivalent**	**H2 blocker; famotidine or equivalent**	**Dexamethasone or equivalent[c]**
Dose Formulation	Ampule	Ampule	Tablet	Tablet	Tablet	Ampule
Unit Dose Strength(s) / Dosage Level(s)	0.05, 0.10, and 0.20 single dose during in SAD cohorts and Q2W or Q4W in RD cohorts[d]	-	500 mg (paracetamol /acetaminophen); 400-800 mg (ibuprofen)	10 mg for cetirizine	20 mg for famotidine	10 mg for IV dexamethasone
Route of Administration	IV infusion	IV infusion	Oral	Oral	Oral	IV infusion
Use	Experimental	Placebo	Premedication[e]	Premedication[e]	Premedication[e]	Rescue medication, or premedication for subsequent infusions if used as rescue medication previously

[a] Regardless of assigned treatment (UX053 or placebo), all subjects in the DB-RD cohort will also be asked to maintain a diet based on expert recommendations for adults with GSD III throughout the study (Table 2).

[b] Paracetamol/acetaminophen is preferred over ibuprofen.

[c] Dexamethasone should be the first-in-line rescue medication; alternative or additional rescue medications are allowed, if necessary, at the discretion of the Investigator. The Medical Monitor must be notified if any rescue medication is used.

[d] Additional cohort(s) at dose levels between 0.05 mg/kg to 0.30 mg/kg may also be included pending review of safety data.

[e] Premedications will be administered to all subjects, including subjects receiving placebo. At the discretion of the Investigator, with input from Ultragenyx and the DMC, and based on the emerging safety profile of UX053, additional premedications and rescue medications may be used to reduce the risk and severity of immune reactions in subjects in RD cohorts. Premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications in patients with GSD III.

DB, double-blind; DMC, Data Monitoring Committee; GSD, Glycogen Storage Disease; IV, intravenous; Q2W, every 2 weeks; Q4W, every 4 weeks; RD, Repeat Dose; SAD, Single Ascending Dose.

In each SAD cohort, subjects will be dosed sequentially with review of safety laboratory data from the 24-hour time point prior to dosing of the next subject. Within SAD cohorts, dosing of individual subjects will occur at a minimum interval of 72 hours. For example, the second subject in a cohort receives their first dose after a minimum of 72 hours after the first subject in the same cohort received their first dose. Subjects within a given RD cohort can be dosed simultaneously. After the first dose, DB-RD subjects maintain a Q2W dosing regimen and OL-RD subjects maintain a Q4W dosing regimen.

Subjects may receive COVD-19 vaccines during participation in the study. To facilitate discrimination of UX053 IRRs and adverse effects of vaccine administration, vaccines should not be administered within 48 hours after the most recent administration of UX053 and until safety labs obtained have been reviewed and the subject has been assessed for any possible hypersensitivity reaction to the most recent dose of UX053 (Section 9.1.6).

For the DB-RD cohort, the subjects, Investigators, and site staff will be blinded to the treatment. Due to the difference in the opacity of UX053 and placebo, an unblinded pharmacist will prepare the investigational product (IP) and an unblinded nurse will prepare the infusion before masking the infusion syringe and tubing with colored covers.

The full schedules of events for the SAD, OL-RD and DB-RD, cohorts are listed in Table 4, Table 5, and Table 6, respectively. Additional details regarding the timing of assessments before, during, and following treatment infusion are provided in Table 7 for vital signs and Table 8 for PK samples.

Although they are eligible, it is expected that few patients with GSD IIIb, IIIc, or IIId will enroll. Subjects with GSD IIIb/c/d will participate in all assessments, including muscle imaging and function tests, and will be included in all safety analyses. Sensitivity analyses for cardiac and skeletal muscle endpoints may exclude patients with GSD IIIb/c/d as described in the SAP.

Future protocol amendments may occur to include pediatric subjects with GSD III or the addition of an open-label extension for all subjects after an interim review of safety, PK, and PD data.

Number of Sites: Approximately 10 sites globally will participate.

Number of Subjects: Approximately 18 adult subjects are planned to be enrolled. Additional subjects may be enrolled to further characterize safety.

Population:

Each subject must meet the inclusion criteria listed below at screening (ISV for DB-RD cohorts) or rescreening (OL-RD cohorts) and baseline (Day 0 for SAD cohorts or Week 0 in all RD cohorts), as applicable, to be enrolled in the study:

1. Confirmed diagnosis of GSD III (all subtypes) based on pathogenic mutations in the amylo-α-1,6-glucosidase 4-alpha-glucanotransferase (*AGL*) gene on both alleles or glycogen debranching enzyme (GDE) deficiency based on biopsy of liver, muscle, or fibroblasts
2. History of any of the following:
    a. Severe hypoglycemia, defined as neuroglycopenia (eg, altered mental status, seizure, dizziness, slurred speech, blurry vision, abnormal behavior, perioral paresthesia,

requiring intervention by a caregiver) or blood glucose < 54 mg/dL (3 mmol/L) within the last year
   b. ≥ 2 incidents of symptomatic hypoglycemia (defined as blood glucose < 70 mg/dL [3.9 mmol/L] if measured at the time of symptoms) within the last year, despite nutrition management
   c. Ongoing liver injury, defined as alanine aminotransferase (ALT) > 2.5x the upper limit of normal (ULN) within the last year

3. For treatment-naïve subjects (SAD and DB-RD cohorts), alanine aminotransferase (ALT) ≤ 5x the upper limit of normal (ULN) during the 3 months prior to the Baseline Visit.
4. Males or females ≥ 18 years of age
5. For subjects enrolling into DB-RD cohorts, willing and able to demonstrate nutrition stability based on nutrition guidelines for adults with GSD III (Table 2)
6. Willing and able to provide access to medical records surrounding medical treatment that occurred prior to enrollment and during the study
7. Willing and able to provide written informed consent, or in the case of adult subjects with cognitive limitation, provide written assent (if required) and written informed consent by a legally authorized representative after the nature of the study has been explained and prior to any test procedures or assessments
8. Females of childbearing potential must have a negative pregnancy test at screening (and rescreening, as applicable) and be willing to have additional pregnancy tests during the study. Subjects of child-bearing potential or males who are sexually active with partners of child-bearing potential must consent to use a highly effective contraceptive method, as described in Appendix 4, from the Period following the signing of the informed consent through 30 days after last dose of study drug
9. For subjects rescreening into OL-RD cohorts after treatment with UX053 in a SAD cohort, subjects must meet the following criteria:
   a. If a significant rise in ALT occurs after the prior dose, ALT should show a decreasing trend toward the subject's baseline value
   b. Total bilirubin is within normal limits
   c. Platelets are within normal limits
   d. International normalized ratio (INR) is within normal limits

Each subject must not meet the exclusion criteria listed below at screening (ISV for DB-RD cohorts) and baseline (Day 0 for SAD cohorts or Week 0 in all RD cohorts), as applicable, to be enrolled in the study:

1. History of liver transplant, including hepatocyte cell therapy/transplant, or active listing for liver transplant
2. History of cirrhosis, or presence of any of the following:
   a. Total bilirubin ≥ 1.3 mg/dL and INR ≥ 1.3
   b. Evidence of portal hypertension, including, but not limited to the following symptoms splenomegaly, ascites, thrombocytopenia, esophageal varices, or history of hepatic encephalopathy

c. Model for End Stage Liver Disease (MELD) score > 12

3. Current Hepatitis B or C infection or history of chronic Hepatitis B or C infection
4. Severe renal impairment defined as a glomerular filtration rate (GFR) ≤ 29 mL/min (Levey et al., 2005).
5. Any prior history of hepatocellular carcinoma or presence of liver adenoma > 5 cm at the longest diameter or > 3 cm and ≤ 5 cm in size that has an annual growth rate of ≥ 0.5 cm per year
6. Current or history of malignancies in the 3 years prior to the Screening Visit (ISV for RD)
7. Hospitalizations related to GSD III disease between the Screening (ISV for RD) and Baseline Visit
8. Known history of human immunodeficiency virus infection
9. Presence or history of any hypersensitivity reactions requiring medical evaluation and management (including injection/IRRs, such as lymphadenopathy) to UX053, its excipients, or any drug products that contain polysorbate or polyethyleneglycol (PEG). This may include vaccines that contain PEG or polysorbate
10. Significant cardiac disease, including heart failure with New York Heart Association (NYHA) Function Capacity III or IV or Objective Assessment C or D, unstable angina, or ejection fraction (EF) < 35%, or uncontrolled arrhythmia or resistant hypertension (Carey et al., 2018). Mild cardiomyopathy and left ventricular hypertrophy (LVH) are allowed
11. Presence or history of any co-morbid condition or abnormal labs that, in the view of the Investigator, places the subject's safety at risk; places the subject at high risk of poor treatment compliance or not completing the study; or would significantly affect the interpretation of study results
12. Poorly controlled diabetes, defined as the presence of any of the following:
    a. Hemoglobin A1C > 8% (Qaseem et al., 2018)
    b. History of diabetic nephropathy, neuropathy, or retinopathy
    c. History of diabetic ketoacidosis during the past year
13. Poorly controlled hypothyroidism, based on the judgement of the Investigator or Ultragenyx, whichever is most conservative
14. History of chronic coagulopathy, thrombophilia, or disorder of complement activation
15. Use of concomitant medications that alter prothrombin time/international normalized ratio (PT/INR), including warfarin and direct oral anticoagulants (eg, rivaroxaban, apixaban, and edoxaban). Patients who receive medications that affect platelet function, such as aspirin or clopidogrel, are allowed unless they have comorbidities that in the judgment of the Investigator place them at undue risk to participate in the study.

16. Current treatment with long-term immunosuppressive medications. This includes subjects with autoimmune conditions managed with immunosuppressive medications and solid organ transplant recipients.
17. Active tuberculosis requiring treatment in the past 3 years
18. Symptomatic coronavirus disease 2019 (COVID-19) infection
19. History of active alcohol and/or drug abuse that in the Investigator's assessment would impair the subject's ability to comply with the protocol
20. Receipt of only 1 of 2 planned doses of a coronavirus disease 2019 (COVID-19) vaccine. Subjects who have not received a COVID-19 vaccine, and those who have completed COVID-19 vaccination are eligible.
21. Planned surgery, including dental surgeries, during the study
22. Pregnant or breastfeeding or planning to become pregnant (self or partner) at any time during the study
23. Females of childbearing potential with hepatocellular adenoma who are unwilling to use nonhormonal contraception
24. Use of any IP or investigational medical device within 30 days or for IP within 5 half-lives, whichever is longer, prior to screening (or rescreening, as applicable), or requirement for any investigational agent prior to completion of all scheduled study assessments
25. For subjects rescreening into OL-RD cohorts, any of the following after treatment with UX053 in a SAD cohort:
    a. New or worsening symptoms of liver disease (including new or worsening hepatomegaly) along with any increase in transaminase levels
    b. Receipt of any blood product administration (eg, packed red blood cells, platelet, fresh frozen plasma [FFP]) for management of consumptive coagulopathy
    c. An ALT level that is ≥ 8x ULN and > 2x the subject's baseline value in the absence of an alternative explanation

Duration of Subject Participation:

SAD Cohorts

Screening is approximately 2 weeks. After screening and dosing, subjects will be monitored for 90 days after dosing.

OL-RD Cohorts

Subjects who have completed participation in a SAD cohort, consisting of screening and a 90-day Follow-up Period, may enter an OL-RD cohort. These subjects are rescreened for participation in the OL-RD cohorts, which is expected to occur over the course of approximately 3 weeks prior to redosing; eligibility for additional dosing in the OL-RD cohorts can be combined with assessments at the SAD Day 90 Visit. After rescreening, subjects will receive a total of 4 additional doses of UX053, with doses administered Q4W, and will be monitored for 36 weeks (Week 48 Visit) after their last dose of UX053.

DB-RD Cohorts

Screening is approximately 8 to 12 weeks. Due to the staggered nature of the cohorts, subjects may remain in nutrition stabilization during screening for ≥ 6 weeks. After screening, subjects will receive a total of 5 doses of UX053, with doses administered Q2W, and will be monitored for 40 weeks (Week 48 Visit) after the last dose of study drug.

Assessments:

Assessments are described in Section 10, and associated timing for each assessment is provided in Table 4, Table 5, and Table 6 for SAD, OL-RD, and DB-RD cohorts, respectively.

Table 4: Schedule of Events for the Single Ascending Dose Cohorts

Day (D) / Week (W)	Screening[a]	BL	Follow-up Period						EOS I (D90)[b]/ ET[c]
		D0	D1	D4	D7	D14	D21	D28	
Visit Window Relative to Baseline	-	-	-	± 1 day		± 2 days			
Informed consent	X								
Inclusion/exclusion	X	X							
Drug Administration		X							
General Assessments									
General & GSD III-specific medical history	X								
COVID-19 testing	X								
Demographics	X								
Physical exam[d]	X	X[e]	X						X
Vital signs	X	X[f]	X	X	X	X	X	X	X
Height	X								
Weight	X	X[e]							X
Adverse events	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X
Pregnancy	X	X[e]						X	X
ATX95		X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	
AGL mRNA		X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	
Anti-drug antibodies		X[e]				X		X	X
Hepatitis B & C	X								
Hemoglobin A1C	X								
Serum CH50, AH50, sC5b-9, & IL-6		X[h]	X			X			
Plasma fibrinogen & d-dimer		X[h]	X			X			
Hematology	X	X[e]	X	X	X	X	X	X	X
Chemistry[i]	X	X[e]	X	X	X	X	X	X	X
Urinalysis[j]	X	X[e]	X	X	X	X	X	X	X
Serum CRP		X[e]	X	X	X	X	X	X	X
Liver									
Liver MRI[k]	X								
Liver MRS[k]	X								
FibroScan or ultrasound elastography	X								
PT/INR[i]	X	X[e]	X		X				X
Serum HDL & LDL[l]	X	X[e]							

Day (D) / Week (W)	Screening[a]	BL	Follow-up Period						EOS I (D90)[b]/ ET[c]
		D0	D1	D4	D7	D14	D21	D28	
Visit Window Relative to Baseline	-	-	-	± 1 day		± 2 days			
Cardiac									
Serum CK-M/B	X	X[e]							
Plasma BNP	X	X[e]							
Serum troponin I	X	X[e]							
ECG	X	X[e]	X						X
Skeletal Muscle & Strength									
Serum CK	X	X[e]							
HHD	X	X[e]							
Clinician- & Patient-reported Outcomes									
PROMIS questionnaires & SF-36v2		X[e]							
GNEM-FAS expanded version		X[e]							
Nutrition diary	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]
Exploratory Assessments									
Serum ketones	X	X[e]	X	X	X	X	X	X	X
Serum biotinidase	X	X[e]							
CGM[n]	X[n]	X[n]	X[n]	X[n]	X[n]	X[n]	X[n]	X[n]	X[n]
Urine Glc_4[j]	X	X[e]			X				X
AGL variant analysis	X								
WBC, RBC, Serum & Plasma for Future Use[o]		X[e]			X			X	X

Footnotes continue on the following page.

[a] Screening assessments can be done over the course of multiple days. There must be a minimum of 10 days between any assessments that are measured at both the Screening and the BL Visit.

[b] At the Day 90 Visit, marking completion of the Follow-up Period, subjects can be rescreened for entry into the OL-RD cohorts. Subjects must sign informed consent to enter into OL-RD cohorts prior to rescreening. Only EOS I Day 90 Visit assessments are included in the table above; please refer to Table 5 for assessments that should be performed for rescreening for OL-RD. If rescreening for OL-RD occurs in conjunction with the SAD EOS I Day 90 visit, assessments in common should not be duplicated.

[c] In the event a subject has an ET, all efforts will be made to monitor the subject through the end of the study. At minimum, a safety follow-up phone call will occur within the 4 weeks following the subject's last treatment.

[d] Complete physical exam is required at the Screening, BL, and EOS I/ET Visits. Targeted physical exams may be performed at the D1 Visit.

[e] Assessments to be performed prior to dosing. The following assessments can be collected within a week prior to dosing, as long as there is a minimum of 10 days between assessments that are repeated at the Screening Visit and the Baseline Visit: HHD, PROMIS questionnaires, SF-36v2, and GNEM-FAS expanded version.

[f] Refer to Table 7 for the timing of vital sign assessments during dosing.

[g] Refer to Table 8 for details of PK sampling. Please note that sample collections are marked for D1, D4, D7, D14, D21, and D28 in this table (Table 4) corresponding to a sample collection 1, 4, 7, 14, 21, and 28 days after infusion of UX053 on D0 as outlined in the final rows of the first column in Table 8.

[h] Sample to be collected pre-dose and at the end of the infusion. Post-infusion blood samples should be collected in the opposite arm of the infusion to avoid dilution.

[i] Close monitoring is required for subjects who develop elevations in ALT > 3x ULN and > 2x their baseline value, or elevation in ALT > 3x ULN with INR > 1.5. This includes repeating liver enzymes and INR within 48 hours and consideration of additional diagnostic tests such as liver ultrasound (Section 10.1.5).

[j] All efforts should be made for urine collections to be consistently taken from the first morning void.

[k] If feasible, glycoNOE imaging of the liver, may be collected at the time of the liver MRI. Liver MRS are only required at sites with capability. Scanning for liver MRS can be conducted immediately following the liver MRI.

[l] A 3- to 4-hour fasting period is preferred, but not required, prior to blood collection for serum HDL and LDL. All efforts should be made to obtain samples for HDL and LDL consistently for a given subject.

[m] The nutrition diary recordings should be recorded at least 3 consecutive days per week through Screening and for the 3 consecutive days in the week leading up to each subsequent visit.

[n] The subject will receive CGM and HHG devices and will be trained on proper use at the Screening Visit. The CGM device is intended to be worn continuously from the Screening Visit to the EOS I D90/ET Visit. If the CGM device alarm notifies a subject that their blood sugar is abnormally high or low or the subject suspects their blood sugar is abnormally high or low, the subject will need to check their blood sugar with a HHG device that will be provided by Ultragenyx during screening. When CGM data is being collected, all subjects will connect their CGM and HHG devices to a study-provided laptop weekly and upload CGM and HHG data. At remote visits, in conjunction with assessments performed by a home health nurse, the site will contact subjects by telephone or videoconference to confirm the CGM device is properly in use and data was uploaded; answer any questions regarding or help troubleshoot (if necessary) CGM device use; and review CGM data, HHG data (when applicable), and nutrition diary entries; outside of scheduled visits, these telephone calls will occur as needed. At in-clinic visits, a conversation between the site and the subject should occur to ensure CGM compliance and troubleshoot if needed.

[o] Serum and plasma samples will be collected for future biomarker assessments yet to be determined.

Note: White columns indicate in-clinic visits (Screening, BL D0, D1, and EOS I D90/ET). Gray columns indicate visits conducted at home by a home health nurse in conjunction with a phone call as needed (D4, D7, D14, D21, and D28).

Note: Laboratory assessments included in chemistry, hematology, and urinalysis are provided in Appendix 3.

AGL, amylo-α-1,6-glucosidase 4-alpha-glucanotransferase; AH50, alternative complement activity; ALT, alanine aminotransferase; BL, baseline; BNP, B-Type Natriuretic Peptide; CGM, continuous glucose monitor; CH50, classical pathway complement activity; CK, creatinine kinase; CK-M/B, CK-muscle/brain; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; D, day; ECG, electrocardiogram; EOS, end of study; ET, early termination; Glc_4, glucose tetrasaccharide; glycoNOE, glycogen nuclear overhauser enhancement; GNEM-FAS, GNE Myopathy Functional Activities Scale; GSD, glycogen storage disease; HHD, handheld dynamometry; HHG, handheld glucometer; HDL, high density lipoprotein; IL-6, interleukin-6; LDL, low density lipoprotein; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; OL, open-label; PROMIS, Patient-Reported Outcomes Measurement Information System; PBMC, peripheral blood mononuclear cell; PK, pharmacokinetic; PT/INR, prothrombin time/international normalized ratio; RBC, red blood cell; RD, repeat dose; sC5b-9, soluble Complement 5b-9; SF-36v2, Short Form Health Survey 36 Version 2; ULN, upper limit of normal; WBC, white blood cell.

Table 5: Schedule of Events for Open-label Repeat Dose Cohorts

Week (W)	Rescreening[a]			Treatment Period								Follow-up Period					EOS II (W48)/ ET[b]
	ISV[a]	NOV[a]	STV[a]	BL/ W0	W1	W2	W4	W5	W8	W9	W12	W13	W14	W16	W24	W36	
Visit Window Relative to BL/Week 0	-	-	-	-	± 1 day	± 2 days									± 3 days		
Informed consent	X																
Inclusion/exclusion	X			X													
Nutrition optimization		X															
Nutrition stabilization			X														
Drug Administration				X			X		X		X						
General Assessments																	
Physical exam[c]				X[d]			X[d]		X[d]		X[d]			X			X
Vital signs	X			X[e]	X	X	X[e]	X	X[e]	X	X[e]	X	X	X	X	X	X
Weight				X[d]			X[d]		X[d]		X[d]			X			X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy	X[d]			X[d]			X[d]		X[d]		X[d]			X	X	X	X
ATX95				X[f]	X[f]	X[f]	X[f]				X[f]	X[f]	X[f]	X[f]			
AGL mRNA				X[f]	X[f]	X[f]	X[f]				X[f]	X[f]	X[f]	X[f]			
Anti-drug antibodies				X[d]			X[d]		X[d]		X[d]			X	X		X
Hemoglobin A1C	X													X			X
Serum CH50, AH50, sC5b-9, & IL-6				X[g]	X	X	X[g]	X	X[g]	X	X[g]	X					
Plasma fibrinogen & d-dimer				X[g]	X	X	X[g]	X	X[g]	X	X[g]	X					
Hematology	X	X[h]	X	X[d]	X	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Chemistry[i]	X	X[h]	X	X[d]	X	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Urinalysis[j]	X	X[h]	X	X[d]	X	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Serum CRP				X[d]	X	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Liver																	
Liver MRI[k]	X[k]													X			
Liver MRS[k]	X[k]													X			
FibroScan or ultrasound elastography[k]	X[k]													X			
PT/INR[i]	X		X	X[d]	X	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Serum HDL & LDL[l]	X	X[h]	X	X[d]			X[d]		X[d]		X[d]			X	X	X	X

Week (W)	Rescreening[a]			Treatment Period								Follow-up Period					EOS II (W48)/ ET[b]
	ISV[a]	NOV[a]	STV[a]	BL/ W0	W1	W2	W4	W5	W8	W9	W12	W13	W14	W16	W24	W36	
Visit Window Relative to BL/Week 0	-	-	-	-	± 1 day	± 2 days									± 3 days		
Cardiac																	
Serum CK-M/B			X	X[d]			X		X		X			X	X	X	X
Plasma BNP		X[h]	X	X[d]			X		X		X			X	X	X	X
Serum troponin I			X	X[d]			X		X		X			X	X	X	X
ECG	X			X[d]							X						X
Skeletal & Muscle Strength																	
Serum CK	X	X[h]		X[d]			X		X		X			X	X	X	X
HHD				X[d]										X			X
Clinician- & Patient-reported Outcomes																	
PROMIS questionnaires & SF-36v2				X[d]										X			X
GNEM-FAS expanded version				X[d]										X			X
Nutrition diary	X[m]			X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]
Exploratory Assessments																	
CFC and associated labs[n]			X[n]											X[n]			
Capillary ketones[o]	X			X[d]			X[d]		X[d]		X[d]			X[o]			X
CGM[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]
Urine Glc4[j]	X			X[d]	X	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
WBC, RBC, Serum & Plasma for Future Use[q]	X			X[d]	X		X				X			X	X		X

Footnotes continue on the following page.

[a] Subjects must sign informed consent for OL-RD cohorts prior to rescreening procedures. Rescreening for OL-RD can be completed at the SAD EOS 1 Day 90 Visit (Table 4); in this situation, the assessments in common should not be duplicated. The ISV includes 3 consecutive days of nutrition diary collection. A dietician will review these 3 days of data and all nutrition data from the subject's prior participation in a SAD cohort to determine if the subject's diet meets nutritional guidelines and has been stable. If no nutritional changes are needed, subjects can proceed to OL-RD STV as soon as ISV assessments are completed. If nutrition changes are needed, NOVs occur as needed during the following week (approximately 2 weeks after the ISV). After 1 week of nutrition optimization, the subject proceeds to the STV (occurring approximately 2 weeks before the subject's OL-RD Baseline/Week 0 Visit) and thereafter maintains a stable diet as defined by the nutritional guidelines for GSD III. If a subject is unable to achieve an optimized diet within 2 weeks of the ISV, they are still eligible to proceed to the STV; the reason for inability to achieve an optimized diet should be documented and a stable diet should be maintained throughout the rest of the study. Figure 2 provides guidance on which screening visits are required for a given subject. All Rescreening Visits can be done remotely, with the exception of completion of imaging assessments (liver MRI, liver MRS, Fibroscan or ultrasound elastography) which must be completed in person at anytime during rescreening.

[b] In the event a subject has an ET, all efforts will be made to monitor the subject through the end of the study. At minimum, a safety follow-up phone call will occur within the 4 weeks following the subject's last treatment.

[c] Complete physical exam is required at rescreening (SAD EOS I Day 90 Visit), W0, and EOS II W48/ET. Targeted physical exams may be performed at W4, W8, W12, and W16.

[d] Assessments to be performed prior to dosing on the same day as dosing visits or on the day before dosing for visits that include a CFC (ie, W16). When applicable, the following assessments can be collected within a week prior to dosing: HHD, PROMIS questionnaires, SF-36v2, and GNEM-FAS expanded version.

[e] Refer to Table 7 for the timing of vital sign assessments during dosing.

[f] Refer to Table 8 for details of PK sampling. Please note that sample collections are marked for W1, W2, W4, W12, W13, W14, and W16 in this table (Table 5) corresponding to a sample collection 7, 14, and 28 days after infusion of UX053 on W0 and/or W12 as outlined in the final rows of the first column in Table 8; a 28-day post infusion collection is only collected for the W12 assessment for the OL-RD cohorts. PK samples are also collected 24-hours post infusion for OL-RD cohorts as noted in Table 8.

[g] Sample to be collected predose and at the end of the infusion. Postinfusion blood samples should be collected in the opposite arm of the infusion to avoid dilution.

[h] Optional safety assessments to be performed (and repeated as necessary) at the discretion of the Investigator if nutrition changes are made during screening.

[i] Close monitoring is required for subjects who develop elevations in ALT > 3x ULN and > 2x their baseline value, or elevation in ALT > 3x ULN with INR > 1.5. This includes repeating liver enzymes and INR within 48 hours and consideration of additional diagnostic tests such as liver ultrasound (Section 10.1.5).

[j] All efforts should be made for urine collections to be consistently taken from the first morning void.

[k] All imaging assessments (liver MRI, liver MRS, and Fibroscan or ultrasound elastography) can be conducted in person at any time during rescreening or within 1 week prior to the corresponding postbaseline visit. Liver MRS are only required at sites with capability. Scanning for liver MRS can be conducted immediately following the liver MRI. If feasible, glycoNOE imaging of the liver, may be collected at the time of the liver MRI.

[l] A 3- to 4-hour fasting period is preferred, but not required, prior to blood collection for serum HDL and LDL. All efforts should be made to obtain samples for HDL and LDL consistently for a given subject.

[m] Nutrition diary recordings should be recorded at least 3 consecutive days per week through rescreening and for the 3 consecutive days in the week leading up to each subsequent visit. Qualified Investigator site staff should review nutrition diary data at each visit.

[n] CFC is an optional assessment. Please see Section 10.5.1 for a description of the CFC. Cortisol must be measured between 6 and 9 am on the morning of the CFC. The CFC begins after completion of a meal (breakfast for daytime fast or dinner for overnight fast) or associated cornstarch consumption (as applicable). A venous blood sample for measurement of glucose, cortisol, glucagon, insulin, C-peptide, and β-hydroxybutyrate will be collected at the beginning (ie, immediately after completion of the meal or the evening cornstarch, as applicable), at end of the CFC, and 30 minutes after the end of the CFC. During the CFC, venous blood samples for STAT analysis of glucose will be collected through an indwelling catheter every 60 minutes (± 5 minutes) until the glucose level decreases to ≤ 70 mg/dL (≤ 3.9 mmol/L). Thereafter, venous blood glucose is measured approximately every 30 minutes (± 5 minutes) until the end of the CFC. The CFC will end when the subject's glucose level decreases to < 54 mg/dL (< 3.0 mmol/L), when the subject experiences signs and symptoms of hypoglycemia, or the fast reaches 12 hours (daytime fast) or 15 hours (overnight fast) without hypoglycemia, whichever occurs first.

[o] Assessment for capillary ketones does not need to be repeated for visits that include the CFC (ie, W16 Visit).

[p] The CGM is intended to be worn continuously from the ISV to the W24 Visit. The CGM will be worn again for the week prior to the W36 and EOS II W48/ET Visits. If the ISV coincides with the SAD EOS I Day 90 Visit, then the CGM device will be worn continuously through screening in the SAD cohort to Week 24 in the RD cohort. If the CGM device alarm notifies a subject that their blood sugar is abnormally high or low or the subject suspects their blood sugar is abnormally high or low, the subject will need to check their blood sugar with a HHG that will be provided by Ultragenyx during screening. When CGM data is being collected, all subjects will connect their CGM and HHG devices to a study-provided laptop weekly and upload CGM and HHG data on a weekly basis. At remote visits, in conjunction with assessments performed by a home health nurse, the site will contact subjects by telephone or videoconference to

confirm the CGM device is properly in use and data was uploaded; answer any questions regarding or help troubleshoot (if necessary) CGM device use; and review CGM data, HHG data (when applicable), and nutrition diary entries; outside of scheduled visits, these telephone calls will occur as needed. At in-clinic visits, a conversation between the site and the subject should occur to ensure CGM compliance and troubleshoot if needed.

[q] RBC, WBC, serum and plasma samples will be collected for future biomarker assessments yet to be determined.

Note: White columns indicate in-clinic visits (ISV, STV, BL/W0, W4, W8, W12, W16, and EOS II W48/ET). Gray columns indicate visits conducted at home (NOV, W1, W2, W5, W9, W13, W14, W24, and W36).

Note: Laboratory assessments included in chemistry, hematology, and urinalysis are provided in Appendix 3.

AGL, amylo-α-1,6-glucosidase 4-alpha-glucanotransferase; AH50, alternative complement activity; ALT, alanine aminotransferase; BL, baseline; BNP, B-Type Natriuretic Peptide; CFC, controlled fasting challenge; CGM, continuous glucose monitor; CH50, classical pathway complement activity; CK, creatinine kinase; CK-M/B, CK-muscle/brain; CRP, C-reactive protein; ECG, electrocardiogram; EOS, end of study; ET, early termination; Glc4, glucose tetrasaccharide; GNEM-FAS, GNE Myopathy Functional Activities Scale; HDL, high density lipoprotein; HHD, handheld dynamometry; HHG, handheld glucometer; IL-6, interleukin-6; LDL, low density lipoprotein; ISV, Initial Screening Visit; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NOV, Nutrition Optimization Visit; OL, open label; PROMIS, Patient-Reported Outcomes Measurement Information System; PK, pharmacokinetic; PT/INR, prothrombin time/international normalized ratio; RBC, red blood cell; RD, repeat dose; SAD, single ascending dose; sC5b-9, soluble Complement 5b-9; SF-36v2, Short Form Health Survey 36 Version 2; STV, Stabilization Visit; ULN, upper limit of normal; W, Week; WBC, white blood cell.

Table 6: Schedule of Events for Randomized, Double-blind, Placebo-controlled Repeat Dose Cohorts

Week (W)	Screening			Treatment Period									Follow-up Period					EOS III (W48)/ ET[b]
	ISV[a]	NOV[a]	STV[a]	BL/ W0	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W12	W24	W36	
Visit Window Relative to Baseline	-	-	-	-	± 1 day	± 2 days										± 3 days		
Informed consent	X																	
Inclusion/exclusion	X			X														
Nutrition optimization		X																
Nutrition stabilization			X															
Randomization				X														
Drug Administration				X		X		X		X		X						
General Assessments																		
General & GSD III-specific medical history	X																	
COVID-19 testing	X																	
Demographics	X																	
Physical exam[c]	X		X	X[d]		X[d]		X[d]		X[d]		X[d]		X				X
Vital signs	X		X	X[e]	X	X[e]	X	X[e]	X	X[e]	X	X[e]	X	X	X	X	X	X
Height	X			X														
Weight	X		X	X[d]		X[d]		X[d]		X[d]		X[d]		X				X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy	X		X	X[d]				X[d]				X[d]		X		X	X	X
ATX95				X[f]	X[f]	X[f]						X[f]	X[f]	X[f]	X[f]			
AGL mRNA				X[f]	X[f]	X[f]						X[f]	X[f]	X[f]	X[f]			
Anti-drug antibodies				X[d]		X[d]		X[d]		X[d]		X[d]		X		X		X
Hepatitis B & C	X																	
Hemoglobin A1C	X														X			X
Serum CH50, AH50, sC5b-9, & IL-6				X[g]		X[g]		X[g]		X[g]		X[g]	X	X				
Plasma fibrinogen & d-dimer				X[g]		X[g]		X[g]		X[g]		X[g]	X	X				
Hematology	X	X[h]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Chemistry[i]	X	X[h]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Urinalysis[j]	X	X[h]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Serum CRP				X[d]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X

Week (W)	Screening			Treatment Period									Follow-up Period					EOS III (W48)/ ET[b]
	ISV[a]	NOV[a]	STV[a]	BL/ W0	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W12	W24	W36	
Visit Window Relative to Baseline	-	-	-	-	± 1 day	± 2 days										± 3 days		
Liver																		
Liver MRI[k]	X[k]													X				
Liver MRS[k]	X[k]													X				
FibroScan or ultrasound elastography[k]	X[k]													X				
PT/INR[i]	X		X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
Serum HDL & LDL[l]	X	X[h]	X	X[d]										X	X	X	X	X
Cardiac																		
Serum CK-M/B			X	X[d]		X		X		X		X		X	X	X	X	X
Plasma BNP		X[h]	X	X[d]		X		X		X		X		X	X	X	X	X
Serum troponin I			X	X[d]		X		X		X		X		X	X	X	X	X
ECG	X			X[d]								X[d]						X
Skeletal & Muscle Strength																		
Serum CK	X	X[h]	X	X[d]		X		X		X		X		X	X	X	X	X
HHD			X	X[d]										X				X
Clinician- & Patient-reported Outcomes																		
PROMIS questionnaires & SF-36v2				X[d]										X				X
GNEM-FAS expanded version				X[d]										X				X
Nutrition diary	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]	X[m]
Exploratory Assessments																		
CFC and associated labs[n]			X[n]											X[n]				
Capillary ketones[o]	X		X[o]	X[d]		X[d]		X[d]		X[d]		X[d]		X[o]				X
CGM[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]
Urine Glc_4[j]			X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X
AGL variant analysis	X																	
WBC, RBC, Serum & Plasma for Future Use[q]				X[d]	X			X						X		X		X

Footnotes continue on the following page.

[a] The ISV includes a review of the subject's diet. Within the first 2 weeks after the ISV, the Investigator will review nutrition diary data to determine if nutrition optimization is needed. If nutrition optimization is not needed, the STV occurs in clinic approximately 2 weeks after the ISV, and the Baseline/Week 0 Visit occurs approximately 6 weeks after the STV. If nutrition changes are needed, a 2- to 4-week period of nutrition optimization will be performed. The first

NOV occurs as a phone visit approximately 2 weeks after the ISV; during nutrition optimization, NOVs can occur weekly or more frequently as needed. When the Investigator determines that the subject has achieved nutritional optimization the STV is performed. Figure 3 provides a schematic of screening for DB-RD cohorts.

[b] In the event a subject has an ET, all efforts will be made to monitor the subject through the end of the study. At minimum, a safety follow-up phone call will occur within the 4 weeks following the subject's last treatment.

[c] Complete physical exam is required at ISV, BL/W0, W2, and EOS III W48/ET. Targeted physical exams may be performed at STV, W4, W6, W8, and W10.

[d] Assessments to be performed prior to dosing on the same day as dosing visits or on the day before dosing for visits that include a CFC (ie, W10). When applicable, the following assessments can be collected within a week prior to dosing: HHD, PROMIS questionnaires, SF-36v2, and GNEM-FAS expanded version.

[e] Refer to Table 7 for the timing of vital sign assessments during dosing.

[f] Refer to Table 8 for details of PK sampling. Please note that sample collections are marked for W1, W2, W9, W10, and W12 in this table (Table 6) corresponding to a sample collection 7, 14, and 28 days after infusion of UX053 on W0 and/or W8 as outlined in the final rows of the first column in Table 8; a 28-day post infusion collection is only collected for the W8 assessment for DB-RD cohorts. PK samples are also collected 24-hours post infusion as noted in Table 8.

[g] Sample to be collected pre-dose and at the end of the infusion. Post-infusion blood samples should be collected in the opposite arm of the infusion to avoid dilution.

[h] Optional safety assessments to be performed (and repeated as necessary) at the discretion of the Investigator if nutrition changes are made during screening.

[i] Close monitoring is required for subjects who develop elevations in ALT > 3x ULN and > 2x their baseline value, or elevation in ALT > 3x ULN with INR > 1.5. This includes repeating liver enzymes and INR within 48 hours and consideration of additional diagnostic tests such as liver ultrasound (Section 10.1.5).

[j] All efforts should be made for urine collections to be consistently taken from the first morning void.

[k] All imaging assessments (liver MRI, liver MRS, and Fibroscan or ultrasound elastography) can be conducted at any time during screening or within 1 week prior to the corresponding visit. Liver MRS are only required at sites with capability. Scanning for liver MRS can be conducted immediately following the liver MRI. If feasible, glycoNOE imaging of the liver, may be collected at the time of the liver MRI.

[l] A 3- to 4-hour fasting period is preferred, but not required, prior to blood collection for serum HDL and LDL. All efforts should be made to obtain samples for HDL and LDL consistently for a given subject.

[m] Nutrition diary recordings should be recorded at least 3 consecutive days per week through Screening and for the 3 consecutive days in the week leading up to each subsequent visit. Qualified Investigator site staff should review nutrition diary data at each visit.

[n] CFC is an optional assessment. Please see Section 10.5.1 for a description of the CFC. Cortisol must be measured between 6 and 9 am on the morning of the CFC. The CFC begins after completion of a meal (breakfast for daytime fast or dinner for overnight fast) or associated cornstarch consumption (as applicable). A venous blood sample for measurement of glucose, cortisol, glucagon, insulin, C-peptide, and β-hydroxybutyrate will be collected at the beginning (ie, immediately after completion of the meal or the evening cornstarch, as applicable), at end of the CFC, and 30 minutes after the end of the CFC. During the CFC, venous blood samples for STAT analysis of glucose will be collected through an indwelling catheter every 60 minutes (± 5 minutes) until the glucose level decreases to ≤ 70 mg/dL (≤ 3.9 mmol/L). Thereafter, venous blood glucose is measured approximately every 30 minutes (± 5 minutes) until the end of the CFC. The CFC will end when the subject's glucose level decreases to < 54 mg/dL (< 3.0 mmol/L), when the subject experiences signs and symptoms of hypoglycemia, or the fast reaches 12 hours (daytime fast) or 15 hours (overnight fast) without hypoglycemia, whichever occurs first.

[o] Assessment for capillary ketones does not need to be repeated for visits that include the CFC (ie, W10 Visit).

[p] The subject will receive a CGM and HHG device and will be trained on proper use at the ISV. The CGM is intended to be worn continuously from ISV to the W24 Visit. The CGM will be worn again for the week prior to the W36 and EOS III W48/ET Visits. If the CGM device alarm notifies a subject that their blood sugar is abnormally high or low or the subject suspects their blood sugar is abnormally high or low, the subject will need to check their blood sugar

with a HHG that will be provided by Ultragenyx at ISV. When CGM data is being collected, all subjects will connect their CGM and HHG devices to a study-provided laptop weekly and upload CGM and HHG data. At remote visits, in conjunction with assessments performed by a home health nurse, the site will contact subjects by telephone or videoconference to confirm the CGM device is properly in use and data was uploaded; answer any questions regarding or help troubleshoot (if necessary) CGM device use; and review CGM data, HHG data (when applicable), and nutrition diary entries; outside of scheduled visits, these telephone calls will occur as needed. At in-clinic visits, a conversation between the site and the subject should occur to ensure CGM compliance and troubleshoot if needed.

[q] RBC, WBC, serum and plasma samples will be collected for future biomarker assessments yet to be determined.

Note: White columns indicate in-clinic visits (ISV, STV, BL/W0, W2, W4, W6, W8, W10, and EOS III W48/ET). Gray columns indicate visits conducted at home (NOV, W1, W3, W5, W7, W9, W12, W24, and W36). A telephone call is required if NOVs occur, but not for W1 and W9. A visit from a home health nurse is required for W1, W3, W5, W7, W9, W12, W24, and W36, but may not be necessary for NOVs.

Note: Laboratory assessments included in chemistry, hematology, and urinalysis are provided in Appendix 3.

AGL, amylo-α-1,6-glucosidase 4-alpha-glucanotransferase; AH50, alternative complement activity; ALT, alanine aminotransferase; BL, baseline; BNP, B-Type Natriuretic Peptide; CFC, controlled fasting challenge; CGM, continuous glucose monitor; CH50, classical pathway complement activity; CK, creatinine kinase; CK-M/B, CK-muscle/brain; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; DB, double-blind; ECG, electrocardiogram; EOS, end of study; ET, early termination; Glc_4, glucose tetrasaccharide; glycoNOE, glycogen nuclear overhauser enhancement; GNEM-FAS, GNE Myopathy Functional Activities Scale; GSD, glycogen storage disease; HDL, high density lipoprotein; HHD, handheld dynamometry; HHG, handheld glucometer; IL-6, interleukin-6; ISV, Initial Screening Visit; LDL, low density lipoprotein; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NOV, Nutrition Optimization Visit; PROMIS, Patient-Reported Outcomes Measurement Information System; PK, pharmacokinetic; PT/INR, prothrombin time/international normalized ratio; RBC, red blood cell; RD, repeat dose; sC5b-9, soluble Complement 5b-9; SF-36v2, Short Form Health Survey 36 Version 2; STV, Stabilization Visit; ULN, upper limit of normal; W, Week; WBC, white blood cell.

Table 7: Schedule of Vital Sign Assessments at Infusion Visits

Pre-Infusion	
Within 30 min prior to initiating the infusion	X
After initiation of infusion[a]	
15 min (± 5 min)	X
30 min (± 5 min)	X
45 min (± 5 min)	X
1 hr (± 10 min)	X
2 hr (± 10 min)	X
3 hr (± 10 min)	X
4 hr (± 10 min)	X
5 hr (± 5 min)	X
6 hr (± 5 min)	X
7 hr (± 10 min)	X
8 hr (± 10 min)	X
9 hr (± 10 min)	X
10 hr (± 10 min)	X
16 hr (+/- 1hr)	X
24 hr (+/- 2hr)	X
Discharge[b]	X

[a] All post-infusion time points refer to time from the beginning of the infusion.
[b] The discharge vital signs assessment is not required and can be used to record any vital signs taken just prior to discharge.
Note: This schedule applies to every infusion of study drug, regardless of cohort (SAD, DB-RD, or OL-RD)
hr, hour; min, minute; OL, open-label; DB-RD, randomized, double-blind, placebo-controlled; RD, Repeat Dose; SAD, Single Ascending Dose.

Table 8: Schedule of PK Assessments during and following Study Drug Infusion

Study Cohort	**SAD Cohorts**		**RD Cohorts**			
Sample Type	**Blood**	**Plasma**	**Blood**	**Plasma**	**Blood**	**Plasma**
Analyte	***AGL* mRNA**	**ATX95**	***AGL* mRNA**	**ATX95**	***AGL* mRNA**	**ATX95**
Infusion	**Day 0 Infusion**		**DB-RD and OL-RD Week 0 Infusion**		**DB-RD Week 8 Infusion &OL-RD Week 12 Infusion**	
Pre-infusion						
Within 1 hr prior to initiating infusion	X	X	X	X	X	X
After initiation of infusion						
1 hr (± 10 min)[a]	X	X	X	X	X	X
3 hr (± 10 min)	X	X	X	X	X	X
4 hr (± 10 min)[b]	X	X	X	X	X	X
4.5 hr (± 10 min)	X	X	X	X	X	X
5 hr (± 10 min)	X	X	X	X	X	X
6 hr (± 20 min)	X	X	X	X	X	X
8 hr (± 30 min)	X	X	X	X	X	X
10 hr (± 30 min)	X	X	X	X	X	X
24 hr (± 2 hr); Day 1	X	X	X	X	X	X
96 hr (± 24 hr); Day 4	X	X				
168 hr (± 24 hr); Day 7	X	X	X	X	X	X
336 hr (± 48 hr); Day 14	X	X	X[c]	X[c]	X	X
504 hr (± 48 hr); Day 21	X	X				
672 hr (± 48 hr); Day 28	X	X	X[d]	X[d]	X	X

[a] The 1-hour sample during infusion is to be collected prior to the infusion rate change (refer to Pharmacy Manual for additional details regarding the infusion rate).

[b] The 4-hour sample is to be collected at the end of the infusion.

[c] Subjects in the DB-RD cohorts receive study drug at the (Week 2) Day 14 Visit, and therefore, their sample is to be collected predosing. While subjects in the OL-RD cohorts do not receive study drug at the Week 2 (Day 14) Visit, their sample should still be collected within the specified time window.

[d] Sample is to be collected pre-dosing at the Week 4 (Day 28) Visit for the OL-RD cohorts only.

Note: 0 hr is the start of the drug infusion.

Note: If the total infusion time is extended beyond 4 hours, a blood sample should be collected at the new EOI time. If the new EOI time overlaps with a prespecified time point, sampling should continue at the next scheduled time point.

AGL, amylo-α-1,6-glucosidase 4-alpha-glucanotransferase; EOI, end of infusion; hr, hour; min, minute; PK, pharmacokinetic; DB, Randomized Double-Blind Placebo-Controlled; RD, Repeat Dose; OL, Open-Label; SAD, Single Ascending Dose.

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4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition or Explanation
ADA	anti-drug antibody
AE	adverse event
AGL	amylo-α-1,6-glucosidase 4-alpha-glucanotransferase
AH50	alternative complement activity
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ApoE	apolipoprotein E
AST	aspartate aminotransferase
ASTCT	American Society for Transplantation and Cellular Therapy
AUC	area under the concentration-time curve
AUC_{inf}	AUC from time 0 to infinity
AUC_{last}	AUC from time 0 to the last measurable concentration
AUC_{tau}	AUC from time 0 to end of dosing period
BNP	B-type natriuretic peptide
BL	Baseline
BUN	blood urea nitrogen
CBC/diff	complete blood count with differential
CFC	controlled fasting challenge
CGM	continuous glucose monitor
CH50	classical pathway complement activity
CK	creatinine kinase
CK-M/B	CK – muscle/brain
CL	Clearance
C_{max}	maximum observed concentration
COVID-19	coronavirus disease 2019
CRF	case report form
CRP	C-reactive protein
CRS	Cytokine Release Syndrome
CTCAE	Common Terminology Criteria for Adverse Events
CYP2B6	cytochrome P450 2B6
DB	double blind
DMC	Data Monitoring Committee
CCI [redacted]	[redacted]

Abbreviation	Definition or Explanation
DPIA	Data Privacy Impact Assessment
CCI	[redacted]
ECG	Electrocardiogram
EDC	electronic data capture
EF	ejection fraction
EOS	end of study
ET	early termination
EU	European Union
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FDA	Food and Drug Administration
FFP	fresh frozen plasma
FIH	first-in-human
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
GDE	glycogen debranching enzyme
GDPR	General Data Protection Regulation
GFR	glomerular filtration rate
GGT	gamma-glutamyl transferase
Glc_4	glucose tetrasaccharide
glycoNOE	glycogen nuclear overhauser enhancement
GNEM-FAS	GNE Myopathy Functional Activities Scale
GMP	Good Manufacturing Practices
GSD	glycogen storage disease
HDL	high density lipoprotein
HED	human equivalent dose
HEENT	head, eyes, ears, nose, and throat
HHD	handheld dynamometry
HHG	Handheld glucometer
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IL-6	Interleukin-6
IND	Investigational New Drug (application)

Abbreviation	Definition or Explanation
INR	international normalized ratio
IP	investigational product
IRB	Institutional Review Board
IRR	infusion-related reaction
IRT	interactive response technology
ISV	Initial Screening Visit
IV	intravenous
CCI	[redacted]
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LDL-R	low density lipoprotein-receptor
LNP	lipid nanoparticle
LVH	left ventricular hypertrophy
MCH	mean corpuscular hemoglobin
MCHC	MCH concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End Stage Liver Disease
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MTD	maximum tolerated dose
NIAID FAAN	National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network
NOAEL	no-observed adverse effect level
NOV	Nutrition Optimization Visit
NYHA	New York Heart Association
OL	open label
PBMC	peripheral blood mononuclear cell
PD	pharmacodynamic(s)
CCI	[redacted]
PK	pharmacokinetic(s)
PP	Per Protocol
PROMIS	Patient-reported Outcomes Measurement Information System
PT	prothrombin time
Q2W	every 2 weeks

Abbreviation	Definition or Explanation
Q4W	every 4 weeks
R_{AUC}	accumulation ratio (calculated as AUC after repeat dose / AUC after a single dose)
RBC	red blood cells
RD	repeat dose
RT-PCR	real time – polymerase chain reaction
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
sC5b-9	soluble Complement 5b-9
SF-36v2	Short Form Health Survey 36 version 2
SOC	System Organ Class
STV	Stabilization Visit
SUSAR	suspected unexpected serious adverse reaction
$T_{1/2}$	half life
TEAE	treatment-emergent adverse event
T_{last}	time of last measurable concentration
T_{max}	time of maximum observed concentration
ULN	upper limit of normal
US	United States
V_{ss}	volume of distribution at a steady state
WBC	white blood cell
WHO	World Health Organization

Definition of Terms

Investigational Product (IP) is defined as, “A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical study, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use” (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [ICH] Harmonised Tripartite Guideline E6: Guideline for Good Clinical Practice).

The term “study drug” may also be used in place of “investigational product” in the protocol.

5. INTRODUCTION

UX053 is an mRNA-based biologic that is being developed for the treatment of Glycogen Storage Disease (GSD) III. The purpose of this first-in-human (FIH) trial is to investigate the safety, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary efficacy of UX053 in adults with GSD III.

5.1. Overview of Glycogen Storage Disease III

Glycogen Storage Diseases represent a group of metabolic disorders in which there is a deficiency in the function of enzymes required to metabolize glycogen, including synthesis, degradation or regulation of glycogen (Wolfsdorf and Weinstein, 2003). Glycogen is the stored form of glucose, with glycogen formation occurring with carbohydrate loading and glycogen degradation (glycogenolysis) to glucose occurring to provide immediate energy and maintain blood glucose levels. GSD III is a rare, autosomal recessive, metabolic disease, characterized by a broad spectrum of clinical manifestations affecting primarily the liver, cardiac muscle, and skeletal muscle (Kishnani et al., 2010). Other names for GSD III include limit dextrinosis disease, Cori disease, and Forbes disease.

GSD III is caused by mutations in the *AGL (*amylo-alpha-1,6-glucosidase 4-alpha-glucanotransferase) gene, encoding for the glycogen debranching enzyme (GDE) (Sentner et al., 2013). GDE is required for complete enzymatic processing of the branch chain linkage in glycogen, a key step in the degradation of glycogen to glucose. *AGL* mutations lead to accumulation of partially metabolized glycogen (limit dextrins) in tissues and a specific incompletely degraded limit dextrin, glucose tetrasaccharide (Glcα1-6Glcα1-4Glcα1-4Glc [Glc_4]), in the urine (Halaby et al., 2019). Limit dextrin accumulation results in hepatic dysfunction, including hypoglycemia, hepatomegaly, and cirrhosis; skeletal myopathy; cardiomyopathy; and growth impairment (Kishnani et al., 2010).

GSD III is a heterogeneous disorder with variable phenotype depending on the site and functional implication of *AGL* mutation. In turn, clinical characteristics vary depending on the relative extent of liver or muscle involvement, with most patients experiencing consequences of both liver and muscle involvement (Sentner et al., 2016). Patients with GSD III generally present by the age of 1.5 years (Sentner et al., 2016; Van Hoof and Hers, 1967), with hepatomegaly the most common presenting clinical sign. Other hallmarks of the disease include ketotic hypoglycemia with fasting, hyperlipidemia, growth retardation, elevated liver transaminases, and elevated creatine kinase. Some individuals also develop osteoporosis or osteopenia over time (Derks and Smit, 2015). Hepatomegaly may appear to improve with age with a reduced relative glucose requirement; however, it is increasingly recognized that progressive liver cirrhosis and hepatic failure can occur, with some patients developing hepatic adenoma, hepatocellular carcinoma, or end-stage liver cirrhosis (Kishnani et al., 2010). Skeletal and cardiac muscle involvement also varies widely, with reports of asymptomatic cardiomyopathy, symptomatic cardiomyopathy leading to death, ventricular hypertrophy, sudden death due to cardiac arrhythmia, and slowly progressing myopathy with distal, proximal or generalized myopathy.

Four subtypes of GSD III have been described: IIIa, IIIb, IIIc, and IIId. Types IIIa and IIIb are the most common, comprising 85% and 15% of the total incidence, respectively. Types IIIc and IIId are extremely rare, with a combined incidence less than 1% of the GSD III population

(Kishnani et al., 2010). The incidence of GSD III is approximately 1:100,000 in the United States (US), with higher incidences in some populations including the North African Jewish population (~1:5,400) and in the Inuit population in Nunavik, Canada (~1:2,500) (Rousseau-Nepton et al., 2015; Endo et al., 2006; Parvari et al., 1997; Moses et al., 1973).

Currently, there is no approved treatment for GSD III, and symptoms are managed with nutrition intervention, including small, frequent feedings and cornstarch to avoid hypoglycemia in children, a high protein diet, low complex carbohydrates, avoidance of fasting, and avoidance of simple sugars (Kishnani et al., 2010). Current nutrition management is focused on prevention of hypoglycemia and maintenance of glucose levels. Case reports indicate that a strict ketogenic regimen may reduce hypertrophic cardiomyopathy (Valayannopoulos et al., 2011; Dagli et al., 2009). No specific management approach has been shown to address the progressive and debilitating muscle impairment experienced by patients with GSD III. The current treatment approach of nutrition management is supportive, but incomplete, and highlights a need for a targeted therapy that replaces the missing or defective enzyme.

5.2. Overview of UX053 Development

A brief overview of existing information on UX053 is provided below; a comprehensive review of available data, including the structural components of UX053, detailed summaries of the nonclinical studies of UX053, and the potential risks and expected benefits of UX053, is contained in the Investigator's Brochure (IB). Information regarding the storage, handling, and administration of UX053 are provided in Section 9.1.

UX053 consists of an mRNA encoding full-length, human GDE encapsulated in a lipid nanoparticle (LNP), supplied in a sterile solution. CCI [redacted].

Intravenous (IV) administration of UX053 demonstrated improvement in GSD III disease manifestations in *Agl* -/- mice and a dog model of GSD IIIa, both of which recapitulate features of human GSD III. Improvement of disease in these models was demonstrated by sustained reductions in glycogen content in the liver that correlated with increases in GDE protein. Glycogen reduction in skeletal muscles has been observed in GSD IIIa dogs treated with UX053, but only at a very low incidence and with minimal effect. It is hypothesized that a longer duration treatment will be necessary to show benefit in the muscle.

The PK characteristics of UX053, determined by measuring both *AGL* mRNA and ATX95, were evaluated in Sprague-Dawley rats and cynomolgus monkeys. Limited PK was also assessed in GSD IIIa and Beagle dogs. In all species, following a single 1-hour IV infusion of UX053, both *AGL* mRNA and ATX95 displayed a biphasic PK profile which can be generally characterized by a rapid distribution phase within the CCI [redacted] after the start of infusion and a slower terminal elimination phase with a longer half-life thereafter ($T_{1/2}$ in monkey: mRNA CCI [redacted] hours; ATX95 CCI [redacted] hours). Dose-dependent increases in *AGL* mRNA and ATX95 exposure were observed from CCI [redacted] mg/kg and CCI [redacted] mg/kg in rat and monkeys, respectively. CCI [redacted].

Toxicology studies in rats and cynomolgus monkeys indicated dose-dependent effects related to hepatic injury, coagulopathy, fibrinolysis, and complement activation, which are consistent with effects that have been reported for other siRNA or mRNA LNP therapies (Levin, 2019; Sabnis et al., 2018; Sedic et al., 2018; Henry et al., 2016).

This protocol is a FIH study; to date, no clinical investigations of UX053 have been conducted.

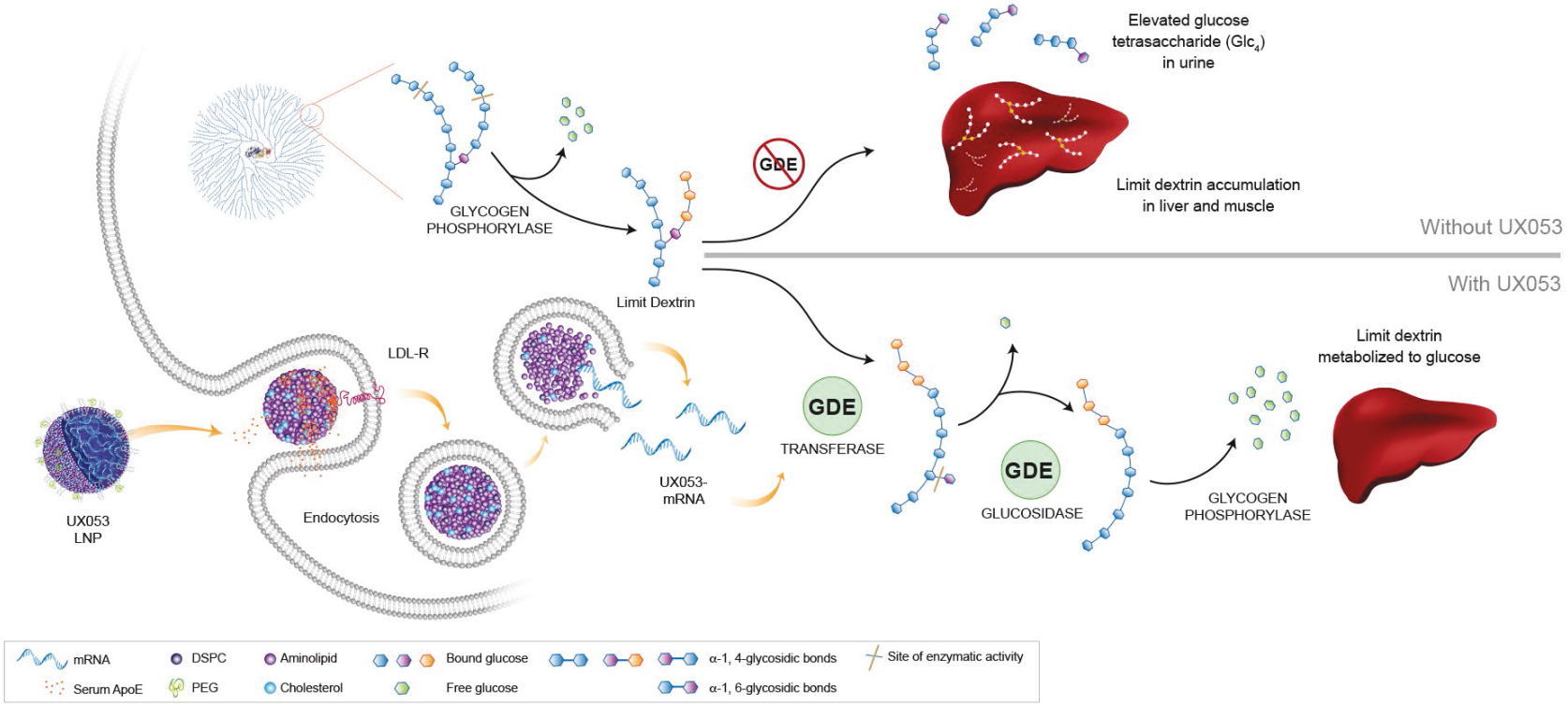
5.3. Rationale for UX053 in Glycogen Storage Disease III

The current treatment approach for GSD III is through nutrition management, including small, frequent feedings and cornstarch to avoid hypoglycemia in children, a high protein diet, low complex carbohydrates, avoidance of fasting, and avoidance of simple sugars (Kishnani et al., 2010). The nutrition management approach demonstrates a limited clinical benefit and does not directly address the underlying pathophysiology of GSD III.

UX053, consisting of an mRNA encoding full-length human GDE encapsulated in an LNP, was designed as a targeted therapy for GSD III. The LNP serves as a delivery system that limits systemic degradation of GDE-encoding mRNA and facilitates its uptake into target cells, predominantly via ApoE receptors (Akinc et al., 2010). In addition, the LNP components promote endosomal escape of the mRNA into the cytoplasm, a necessary step for translation into GDE (Figure 4). The mRNA is codon optimized for expression in human cells, thereby addressing GDE deficiency, the underlying pathophysiology of GSD III. In nonclinical species, UX053-derived GDE is active in the cytoplasm of target cells, where it facilitates glycogen breakdown. GDE expression in target cells is transient because the encoding mRNA is degraded over time by endogenous cellular mechanisms. As briefly described in Section 5.2, UX053 demonstrated improvement in GSD III disease manifestations in nonclinical models. Restoration of GDE activity in patients with GSD III is expected to slow or halt disease progression by restoring glucose homeostasis and reducing liver and muscle injury. Hepatomegaly due to glycogen overload is expected to reverse.

The mRNA-LNP modality was selected for the treatment of GSD III because the LNP facilitates targeted distribution of full-length GDE-encoding mRNA to the liver, and may also facilitate uptake into skeletal and cardiac muscles, thereby enabling sufficient expression of GDE in key target tissues. The transient nature of both mRNA and UX053-derived GDE necessitates periodic repeat dosing of UX053, presenting an opportunity to adjust the dose as needed to attain optimal safety and clinical outcomes. Of note, the size of the human *AGL* mRNA (5023 nucleotides) precludes gene delivery using a viral vector-DNA–based modality (Wu et al., 2010), and traditional enzyme replacement is not feasible with GDE given that it functions in cellular cytoplasm (Solomon and Muro, 2017).

Figure 4: Schematic Representation of UX053 Mechanism of Action



During glycogenolysis, the glycogen phosphorylase enzyme cleaves the peripheral glucosyl residues until 4 residues remain before the α-1,6 branch point. The terminal 3 residues are then transferred to another branch by the transferase component of GDE. The glucosidase component of GDE then cleaves the α-1,6 branch point. When the debranching enzyme is deficient, only the peripheral glucosyl residues can be cleaved and an abnormal glycogen, called limit dextrin, accumulates (Dagli et al., 2009).

ApoE, Apolipoprotein E; GDE, glycogen debranching enzyme; LDL-R, low density lipo-protein receptor; LNP, lipid nanoparticle; CCI [redacted]

5.4. Potential Risks and Benefits

Considering measures to minimize risk to subjects in this study, the potential risks associated with UX053 are justified by the anticipated benefits that may be afforded to subjects.

5.4.1. Potential Risks for UX053

This FIH study was designed to mitigate the known risks associated with siRNA or mRNA LNP therapies and potential risks identified in nonclinical toxicology studies with UX053. Potential risks for UX053 were identified by reviewing data from nonclinical toxicology studies with UX053 and pharmacological class effects from clinical studies with relevant LNP-formulated oligonucleotide therapies, including mRNA-LNP therapies. The potential risks of treatment with UX053 include:

- Hypersensitivity
- Hepatotoxicity
- Consumptive coagulopathy

Hypersensitivity may include infusion-related reactions (IRRs), immune system activation, as well as the development of anti-drug antibodies (ADA) to human GDE, CCI [redacted], or both. Example symptoms related to immune system activation may include, but are not limited to, increased complement activity and cytokine release, elevated body temperature, neutrophilia, and tachycardia.

Hepatotoxicity may be evident by increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), or gamma-glutamyl transferase (GGT).

Consumptive coagulopathy may be evident as prolonged PT/INR, decreased fibrinogen, or unusual bleeding or bruising.

For details on how the FIH study was designed to mitigate the potential risks of UX053, refer to Section 5.5.

5.4.1.1. Potential Risks Identified from Nonclinical Studies

A review of nonclinical studies with UX053 informed the identification of the following potential risks for UX053: hypersensitivity, hepatotoxicity, and consumptive coagulopathy.

Summaries of nonclinical studies with UX053 are provided in the IB. Across species (mouse, rat, dog, and monkey), the key potential risks identified in nonclinical studies were hypersensitivity, consumptive coagulopathy, and hepatotoxicity. The potential risks are likely triggered in part by immune system activation including, complement activation and cytokine release. Clinical signs of complement activation can include fever, tachycardia, hypotension, body aches, nausea and vomiting, rash, and edema.

ADAs to human GDE and CCI [redacted] developed in rats, dogs, and monkeys after repeat administration of UX053. Increasing titers over time coincided with a progressively higher magnitude induction of cytokines and complement activation at later doses in dogs and monkeys (cytokines and complement not measured in rats). The presence of CCI [redacted] ADAs did not have an impact on UX053 mRNA and ATX95 exposure in these studies.

Hepatotoxicity was identified by increases in transaminases and corresponding changes in histopathology. At lower doses, rises in transaminases were transient and reversible. Importantly, these changes are clinically monitorable.

In the nonclinical toxicology studies in rat and cynomolgus monkey, toxicity was observed at doses of CCI higher than the highest potential dose assigned in this FIH study on a mg/kg basis. Dogs, which have been reported as a model with hypersensitivity to nanoparticle formulations (Szebeni et al., 2018; Szebeni et al., 2007), had symptoms of toxicity at CCI mg/kg UX053, which is higher than the highest potential dose in this FIH study (option to dose up to CCI mg/kg). The heightened sensitivity to such reactions in dogs may translate to similar types of findings in patients who experience hypersensitivity (eg, IRRs), and hypersensitivity has been identified as an important potential risk and will be carefully monitored. Toxicology findings from UX053 nonclinical studies across species were consistent with findings for other siRNA or mRNA LNP therapies (Levin, 2019; Sabnis et al., 2018; Sedic et al., 2018; Henry et al., 2016).

5.4.1.2. Potential Risks Identified from Relevant LNP-formulated Oligonucleotide Therapies

A review of clinical studies with similar LNP-formulated oligonucleotide therapies informed the identification hypersensitivity and immune system activation as potential risks for UX053.

Potential class effects were identified by review of data from similar molecules in clinical development and data from clinical studies of patisiran (ALN-TTR02, ONPATTRO), an siRNA LNP treatment approved by the US Food and Drug Administration (FDA) in 2018 for the treatment of polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults (ONPATTRO). IRRs were observed in patients treated with patisiran in clinical trials. IRR symptoms included, but were not limited to: arthralgia or pain (including back, neck, or musculoskeletal pain), flushing (including erythema of face or skin warm), nausea, abdominal pain, dyspnea or cough, chest discomfort or chest pain, headache, rash, chills, dizziness, fatigue, increased heart rate or palpitations, hypotension, hypertension, and facial edema.

It is anticipated that administration of UX053 may induce antibody responses to human GDE and CCI Anti-human GDE antibodies may develop once the full-length human GDE is translated from UX053 mRNA in patients with GSD III. CCI antibodies may develop as a direct response to the CCI present in the LNP, CCI as UX053 (ONPATTRO). CCI ADA will be measured as described in Section 10.1.6.

Available safety data for the following mRNA LNP therapeutics were also reviewed to identify potential pharmacological class effects: CCI (mRNA for OTC deficiency); mRNA-1273 (mRNA-based vaccine for SARs-CoV-2); and H10N8 and H7N9 (mRNA-based vaccines against influenza). As previously noted, CCI

After completion of the 3 dose-escalation cohorts with CCI up to 0.3 mg/kg in a Phase 1 study in healthy volunteers, all adverse events (AEs) were mild to moderate and no serious adverse events (SAEs) occurred (Arcturus Therapeutics, 2020).

Although the structure of the LNP is similar to UX053, mRNA-1273 is administered intramuscularly and only 2 doses are provided (Jackson et al., 2020). In the Phase 1 study, AEs reported in more than half of the 45 subjects following treatment with mRNA-1273 included fatigue, chills, headache, myalgia, and pain at the injection site. One subject in the Phase 1 study experienced Grade 3 (severe) incidents of syncope and lightheadedness on the day of the second vaccination; these events were considered related to treatment and resolved the following day. Adverse events were more common after the second vaccination. No pattern of concern emerged among safety laboratory values. Preliminary analysis of the Phase 3 study was consistent with findings from the Phase 1 study (Moderna Therapeutics, 2020). Grade 3 (severe) AEs occurring in > 2% of subjects (> 30,000 subjects included in the analysis) were injection site pain, fatigue, myalgia, arthralgia, headache, pain, and erythema/redness at the injection site.

In a Phase 1 trial with H10N8 and H7N9, LNP-based mRNA vaccines against influenza, AEs included injection site pain and swelling, erythema, headache, fatigue, myalgia, arthralgia, nausea, and fever (Feldman et al., 2019).

A similar spectrum of AEs has been reported for other relevant IV LNP-based nucleic acid therapies in clinical trials: ALN-TTR01 (siRNA also evaluated for treatment of hereditary transthyretin mediated amyloidosis) (Coelho et al., 2013), ALN-PCS02 (siRNA for hypercholesterolemia) (Fitzgerald et al., 2014), ARB-1467 (siRNA targeting hepatitis B virus) (Streinu-Cercel et al., 2017), and BMS-986263 (siRNA for HSP47 to treat liver and pulmonary fibrosis) (Sakamoto et al., 2018; Soule et al., 2018). The most common safety finding among these agents was infusion reactions which were in some cases reported to be mitigated by slowing the infusion rate. Headache, flushing, edema, and flu-like symptoms were commonly reported AEs in some of these studies.

5.4.2. Potential Risks of Premedication

The premedication regimen for this study is based on the premedication regimen established for the approved siRNA LNP therapy, patisiran (ONPATTRO). Premedications recommended for patisiran are IV corticosteroids (dexamethasone 10 mg, or equivalent), acetaminophen, IV H1 blocker (eg, diphenhydramine 50 mg or equivalent), and an IV H2 blocker (eg, ranitidine 50 mg or equivalent). IV diphenhydramine is associated with drowsiness, dizziness, constipation, upset stomach, blurred vision, and dry mouth, possibly due to anticholinergic effects. Instead of IV diphenhydramine, premedication for UX053 includes a more selective oral antihistamine, cetirizine 10 mg, or equivalent, which is less sedating. Premedication for UX053 also includes an oral H2 blocker (famotidine 20 mg or equivalent). The safety profiles of oral acetaminophen/paracetamol, cetirizine, and famotidine have been well characterized. These formulations are available without a prescription in all participating countries. At the discretion of the Investigator, with input from Ultragenyx and the Data Monitoring Committee (DMC), and based on the emerging safety profile of UX053, additional premedications and rescue medications may be used to reduce the risk and severity of immune reactions, including IRR, in repeat dose (RD) cohorts. Premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications in patients with GSD III.

To minimize potentially unnecessary chronic exposure to corticosteroids, dexamethasone will be used as a rescue agent for subjects who develop a hypersensitivity reaction ≥ Grade 2 in severity suspected to be due to immune activation (Section 8.3.2 and Section 9.1.1), and should be

considered as a premedication for subsequent infusions for that subject after discussion with the Medical Monitor. If a subject develops an infusion-related reaction that meets diagnostic criteria for anaphylaxis (Section 8.3.3), epinephrine should be administered as initial rescue medication.

5.4.3. Potential Risks for Study Participation

Physical injury associated with study assessments and procedures is also a potential risk. This includes discomfort, bleeding, bruising from phlebotomy and local skin irritation from the continuous glucose monitor (CGM) or electrocardiogram (ECG) electrodes. There is also the potential risk of injury during muscle strength tests.

5.4.4. Potential Benefits

Nonclinical studies suggest that treatment with UX053 may improve clinical manifestations of GSD III, including increases in functional GDE levels and reductions in glycogen content in the liver. A more detailed summary of the potential benefits observed in nonclinical studies of UX053 are provided in the IB. Subjects with GSD III participating in this FIH study may experience a transient, but meaningful, benefit from even a single dose of UX053. Nonclinical studies suggest that treatment with UX053 may reduce hepatomegaly, hypoglycemia, and liver damage. Secondary beneficial effects may include improvements in glucose metabolic availability during exercise, which may have indirect benefits on muscle injury and function. Due to the short half-life of the mRNA, such benefits are likely to be lost after discontinuation of UX053 treatment. To maximize potential benefit to study subjects, those who complete the SAD 90-day follow-up may be rescreened to enter open-label (OL)-RD cohorts, in which they will receive 4 additional doses of UX053 at monthly intervals. Assuming the clinical development of UX053 continues, subjects who participate in this FIH study will also be eligible for additional treatment within a long-term open-label extension study.

Currently, there is no approved treatment for GSD III, and symptoms are managed with diet. Subjects may benefit from participation in this study by receiving enhanced disease management from clinicians with expertise treating GSD III. Subjects in the OL-RD and double-blind (DB)-RD cohorts, including those randomized to placebo may benefit from nutrition optimization during screening and dietary monitoring throughout the study (Section 7.1). Subjects may also directly benefit by having previously undetected disease manifestations identified during the study assessments.

Participation in this study will help to provide a better understanding of the disease-specific features of GSD III and support the development of therapies for GSD III, including UX053.

5.5. Risk Minimization

Several components of this FIH study are designed to mitigate the potential risks of study participation, including implementation of a DMC, 72-hour intervals between dosing of individual subjects within a given SAD cohort, a cautious dose escalation scheme, slow drug infusion rate, prespecified premedications, availability of rescue medications, continuous cardiorespiratory monitoring during infusions, and 24-hour observation post infusion to monitor for post infusion effects.

Dose escalation will occur only after safety data reviews by the DMC. The DMC consists of a panel of independent medical and scientific experts (Section 7.1 and Section 11.12). Standard

safety laboratory tests are collected immediately prior to dosing (all cohorts) and again approximately 24 hours after dosing and at Day 4 (SAD cohorts) and Day 7 (both SAD and RD cohorts) (Section 10.1.5). In addition, characterization of complement activation through classical and alternative pathways, cytokine release, fibrinogen, and d-dimer will be assessed predose and at the end of each infusion. Potential risks of UX053 treatment identified from available nonclinical data with UX053 and known risks associated with siRNA or mRNA LNP therapies (Section 5.4.1) will be monitored throughout participation in the SAD and RD cohorts. Subjects will be monitored in an inpatient or observational unit for 24 hours after the end of each infusion.

The starting dose of UX053, 0.05 mg/kg, is approximately CCI below the human equivalent dose (HED) for the no-observed adverse effect level (NOAEL) for repeat dosing in dogs, approximately CCI below the HED for the NOAEL for repeat dosing in rats, and approximately CCI below the HED for the NOAEL for repeat dosing in cynomolgus monkeys using CCI scaling to calculate HED. The relative dose increase becomes smaller with each subsequent cohort: after a CCI dose increase from Cohorts 1S to 2S to 3S, there is a CCI dose increase between cohorts 3S and 4S. For RD cohorts, sequential doses may be reduced for individual subjects or for entire cohorts based on emerging safety findings.

Subjects in SAD cohorts will be dosed at least 72 hours apart in each cohort, minimizing exposure to subjects if an early safety signal occurs. At least 2 weeks of safety data from at least 2 subjects in each SAD cohort will be reviewed prior to making a decision to proceed with the next cohort. Either at least 6 weeks of safety data from at least 4 subjects in a DB-RD cohort or 12 weeks of safety data from at least 3 subjects in an OL-RD cohort (whichever is available first) will be reviewed prior to making a decision to proceed with repeat dosing at the next dose level. The intervals between subjects and cohorts dosing were determined based on nonclinical studies, which showed that single-dose toxicities generally occurred soon after dosing, and that repeat-dose toxicities were observed after CCI
A detailed summary of nonclinical findings with UX053 is provided in the IB.

Study drug will be administered as an infusion over the course of at least 4 hours, with a slower rate of infusion for the first hour to minimize the risk of hypersensitivity or anaphylactoid-type reactions. The infusion rate may be slowed to minimize the risk of IRRs in individual subjects. Subjects will receive premedication at least 1 hour prior to infusion.

If a subject develops an infusion-related reaction that meets diagnostic criteria for anaphylaxis (Section 8.3.3), epinephrine should be administered as initial rescue medication. For all other hypersensitivity reactions ≥ Grade 2 in severity suspected to be due to immune activation, dexamethasone should be the initial rescue medication (Section 9.5). The Medical Monitor must be notified if any rescue medication is used. If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor.

A similar premedication regimen that includes dexamethasone is used for patisiran, a siRNA LNP treatment approved by the FDA in 2018 for the treatment of polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults (ONPATTRO).

Testing for ADA to both human GDE and CCI [redacted] will be performed, and titers will be obtained. ADA testing will be performed at appropriate frequencies (schedule provided in Table 4, Table 5, and Table 6), based on FDA guidance (FDA, 2019b; FDA, 2014). Back-up serum samples will be banked and stored frozen for future use if further characterization of the immune response is required.

To minimize risk of coronavirus disease 2019 (COVID-19) exposure, subjects will be encouraged to observe social distancing, wear face masks/coverings, and avoid social gatherings during the conduct of the clinical trial and site visits, as long as country and local health authorities recommend. Subjects should follow local public health guidance and Institutional Review Board (IRB) recommendations. Guidance on potential changes to the protocol due to COVID-19 is in Section 7.1.5. Guidance on COVID-19 vaccination is in Section 9.1.6.

Subjects will be continually monitored by trained site personnel during study procedures, including laboratory collection and handheld dynamometry (HHD), to ensure safety and minimize risk of injury.

6. OBJECTIVES AND ENDPOINTS

Table 9: Study Objectives and Endpoints

OBJECTIVES	ENDPOINTS
Primary	
Evaluate the safety of UX053 in adults with GSD III	The incidence and severity of TEAEs, serious TEAEs, and related TEAEs in the SAD and RD cohorts
Secondary	
Characterize the PK of UX053 in adults with GSD III	PK parameters of *AGL* mRNA and ATX95, which may include T_{max}, C_{max}, AUC_{last}, AUC_{inf}, AUC_{tau} (RD cohorts only), R_{AUC} (RD cohorts only), T_{last}, $T_{1/2}$, CL, V_{ss}

AGL, amylo-α-1,6-glucosidase 4-alpha-glucanotransferase; AUC, area under the concentration-time curve; AUC_{inf}, AUC from time 0 to infinity; AUC_{last}, AUC from time 0 to the last measurable concentration; AUC_{tau}, AUC from time 0 to end of dosing period; CL, clearance; C_{max}, maximum observed concentration; PK, pharmacokinetics; R_{AUC}, accumulation ratio (calculated as AUC after repeat dose / AUC after a single dose); RD, Repeat Dose; SAD, Single Ascending Dose; $T_{1/2}$, half life; TEAE, treatment-emergent adverse events; T_{last}, time of last measurable concentration; T_{max}, time of maximum observed concentration; V_{ss}, volume of distribution at a steady state.

Tertiary CCI [redacted] and exploratory objectives CCI [redacted] and their corresponding endpoints are provided in the Statistical Analysis Plan (SAP).

7. INVESTIGATIONAL PLAN

7.1. Study Design

UX053-CL101 is a phase 1/2 FIH study to evaluate the safety, tolerability, and PK of SAD and RD of UX053 in patients with GSD III (Figure 1). The SAD cohorts will be OL. There will be 2 types of RD cohorts: 1) OL-RD and 2) randomized, DB, and placebo-controlled RD (referred to as DB-RD). Treatment-naïve subjects enter the SAD and DB-RD cohorts. Subjects in the SAD cohorts who complete the 90-day Follow-up Period can enter OL-RD cohorts. Details for each of these cohorts are described below.

Single Ascending Dose Cohorts

The SAD cohorts evaluate safety and PK of single doses of UX053 in treatment-naïve subjects. All subjects in the SAD cohorts will undergo a Screening Visit prior to their Baseline Visit. There are 3 planned SAD cohorts, each consisting of at least 2 subjects, all of whom will receive open-label UX053 (Figure 1). The planned dose escalation will proceed from 0.05 mg/kg in Cohort 1S, to 0.10 mg/kg in Cohort 2S, and to 0.20 mg/kg in Cohort 3S. An independent DMC will review at least 2 weeks of safety data for at least 2 subjects within a SAD cohort and the cumulative data from all subjects in prior dosing cohorts (when applicable) before deciding to proceed with the next SAD cohort.

Each subject in the SAD cohorts will be followed for a follow-up of 90 days after dosing. At the SAD Day 90 Visit, marking completion of the follow-up, subjects can be rescreened for entry into the OL-RD cohorts described below.

Open-Label Repeat Dosing Cohorts (Extension for SAD Cohorts)

The OL-RD cohorts serve as a short-term extension for subjects who participated in a SAD cohort. The OL-RD cohorts evaluate the safety and PK of 4 additional OL doses of UX053 in subjects who received a single dose of UX053 in a SAD cohort and completed the 90-day Follow-up Period. Subjects from the SAD cohorts are rescreened for participation in the OL-RD cohorts; eligibility for additional dosing in the OL-RD cohorts can be combined with assessments at the SAD Day 90 Visit.

During rescreening, a nutrition assessment will be completed at an Initial Screening Visit (ISV) to determine if the subject's diet meets the nutritional guidelines for adults with GSD III based on expert recommendations (Table 2). OL-RD subjects will proceed through rescreening as shown in Figure 2 based on whether they meet these nutritional guidelines.

The first day of the ISV (which may coincide with the SAD Day 90 Visit) is followed by 3 consecutive days of nutrition diary collection. If a subject's diet during participation in a SAD cohort meets nutritional guidelines, and continues to do so at the ISV, the subject can proceed directly to the OL-RD Nutrition Stabilization Visit (STV) as soon as the ISV assessments are completed.

If the subject's diet does not meet nutritional guidelines, a Nutrition Optimization Visit (NOV) is performed. At this visit, nutritional counseling is provided to the subject on dietary changes needed to comply with GSD III-specific nutrition guidelines (Table 2). Nutrition diary and continuous glucose monitor (CGM) data will be reviewed by the Investigator and safety

laboratory tests may be obtained at the discretion of the Investigator. After 1 week of nutrition optimization (approximately 2 weeks after the ISV), the subject proceeds to the STV (occurring approximately 2 weeks before the subject's OL-RD Baseline/Week 0 Visit) and thereafter maintains a stable diet as defined by the nutritional guidelines for GSD III (Table 2) for the remainder of the study. If an OL-RD subject is unable to achieve an optimized diet, they are still eligible to proceed to the STV. The reason for the inability to achieve an optimized diet should be documented and the subject should make no substantial nutrition changes for the remainder of the study.

For selected sites and subjects, the OL-RD STV includes an optional controlled fasting challenge (CFC). Prior to the CFC, a morning cortisol is obtained between 6 and 9 am. The CFC is conducted during the subject's stay in the hospital, usually overnight, but can be conducted during the day. For the same sites and subjects, another optional CFC occurs at the Week 16 Visit; only subjects who completed the optional CFC at STV complete the CFC at Week 16. Additional details regarding the CFC are in Section 10.5.1.

Beginning with the OL-RD Baseline/Week 0 Visit, subjects in the OL-RD cohorts will receive 4 additional OL doses of UX053 Q4W at the same or lower dose than they received in the SAD cohorts, based on ongoing assessment of safety data.

Randomized, Double-Blind, and Placebo Controlled Cohorts

The DB-RD cohorts will evaluate the safety and PK of 5 doses of UX053 or placebo in treatment-naïve subjects. All DB-RD subjects will participate in a Screening Period (approximately 8 to 12 weeks) (Figure 3), in which subjects will be screened for study eligibility and assessed for the need to optimize their diet based on nutrition guidelines for adults with GSD III based on expert recommendations (Table 2). Within the first 2 weeks after the ISV, the Investigator will review nutrition diary data to determine if nutrition optimization is needed. If nutrition optimization is not needed, the STV occurs in clinic approximately 2 weeks after the ISV, and the Baseline/Week 0 Visit occurs approximately 4 weeks after the STV, to demonstrate 6 weeks of nutrition stability.

If nutrition changes are needed, a 2- to 4-week period of nutrition optimization will be performed to comply with GSD III-specific nutrition guidelines (Table 2). In this situation, the first NOV occurs as a phone visit approximately 2 weeks after the ISV. During nutrition optimization, NOV phone visits occur weekly or more frequently as needed; nutrition diary and CGM data are reviewed; and safety laboratory tests may be obtained at the discretion of the Investigator. Nutritional counseling is provided to the subject. When the Investigator determines that the subject has achieved nutritional optimization the STV is performed.

The Baseline/Week 0 Visit occurs as least 6 weeks after the STV. Due to the staggered nature of the cohorts (described below), a subject may be in nutrition stabilization for longer than 6 weeks before their Baseline Visit; in such instances, the total duration of the Screening Period may exceed 12 weeks. Nutrition guidance and monitoring for this study was established with available health authority guidance, and greater detail is available in the Study Reference Manual.

After the STV, no substantial nutrition changes should be made through the remainder of the study, notwithstanding temporary changes due to illness.

For selected sites and subjects, the DB-RD STV includes an optional controlled fasting challenge (CFC). Prior to the CFC, a morning cortisol is obtained between 6 and 9 am. The CFC is conducted during the subject's stay in the hospital, usually overnight, but can be conducted during the day. For the same sites and subjects, another optional CFC occurs at the Week 10 Visit; only subjects who completed the optional CFC at STV complete the CFC at Week 10. Additional details regarding the CFC are in Section 10.5.1.

In DB-RD cohorts, treatment-naive subjects will be randomized 3:1 to UX053 or placebo in ascending dose cohorts (4 subjects per cohort) (Figure 1). DB-RD subjects will receive study drug Q2W; a total of 5 doses of study drug are planned. The planned dose levels are 0.05 mg/kg in cohort DB-1R, 0.10 mg/kg in cohort DB-2R, and 0.20 mg/kg in cohort DB-3R.

Initiation of RD Cohorts (Both OL-RD and DB-RD)

Initiation of RD cohorts is as follows:

- Initiation of Cohort DB-1R and OL-1R will be guided by DMC review of at least 2 weeks of safety data from at least 2 Cohort 2S subjects and any available data from all subjects in prior and ongoing cohorts.
- Initiation of Cohort OL-2R and DB-2R will be guided by DMC review of either 1) at least 6 weeks of safety data from at least 4 subjects within Cohort DB-1R or 2) at least 12 weeks of safety data from at least 3 subjects within Cohort OL-1R (whichever is available first). In both of these scenarios, all available data from all subjects in prior and ongoing cohorts will also be reviewed.
- Initiation of Cohort OL-3R and Cohort DB-3R will be guided by DMC review of either 1) at least 6 weeks of safety data from at least 4 subjects within Cohort DB-2R or 2) at least 12 weeks of safety data from at least 3 subjects within Cohort OL-2R (whichever is available first). In both of these scenarios, all available data from all subjects in prior and ongoing cohorts will also be reviewed.

Cohort- and Subject-level Dose Selection and Modification for All Cohorts (SAD, OL-RD, and DB-RD)

At any time Ultragenyx may decide not to initiate a planned dose cohort. Dose levels for each cohort may be altered depending on safety, PK, and/or PD findings from prior cohorts. If needed to evaluate safety and dose-response relationships, additional cohorts may be added to test doses up to 0.30 mg/kg or additional subjects may be added to a cohort at the discretion of Ultragenyx to further characterize safety, PK, and PD effects, with input from the DMC. In every case, the safety of a single dose will be evaluated prior to repeat doses at the same dose level.

If a subject in an RD cohort develops a treatment-emergent adverse event (TEAE)/serious TEAE ≥ Grade 3 that is considered by the Investigator to be related to study drug or an intolerable TEAE, subject-level dose reductions are allowed in consultation with the Medical Monitor. Ultragenyx will notify the DMC immediately if any subject experiences an event that satisfies subject-level stopping criteria (Section 8.3.2) or study-level stopping criteria (Section 8.3.3).

Glucose and Nutrition Data Collection by Study Subjects

At the Screening Visit for the SAD cohorts and ISV for the DB-RD cohorts, all subjects will receive CGM and handheld glucometer (HHG) devices and will be trained on the proper use of

these devices. Subjects will also be trained on completion of the nutrition diary During screening. Subjects entering the OL-RD cohorts do not need to be retrained if compliance was acceptable during participation in the SAD cohorts. When CGM data is being collected, all subjects will connect their CGM and HHG devices to a study-provided laptop weekly and upload CGM and HHG data. At remote visits, in conjunction with assessments performed by a home health nurse, the site will contact subjects by telephone or videoconference to confirm the CGM device is properly in use and data was uploaded; answer any questions regarding or help troubleshoot (if necessary) CGM device use; and review CGM data, HHG data (when applicable), and nutrition diary entries; outside of scheduled visits, these telephone calls will occur as needed. At in-clinic visits, a conversation between the site and the subject should occur to ensure CGM compliance and troubleshoot if needed.

Drug Administration, Dose Interval, and Blinding

UX053 will be administered as an intravenous (IV) infusion over the course of at least 4 hours, with a slower rate of infusion for the first hour to minimize the risk of hypersensitivity or anaphylactoid-type reactions (refer to the Pharmacy Manual for additional infusion details) (Table 3 and Section 9). The infusion rate may be slowed to minimize the risk of IRRs in individual subjects.

Premedication and Rescue Medication

All subjects, including those treated with placebo, will receive premedication at least 1 hour prior to the infusion, consisting of oral paracetamol/acetaminophen (500 mg) or ibuprofen (400 to 800 mg), an H2 blocker (eg, famotidine 20 mg or equivalent dose of another H2 blocker), and an H1 blocker (eg, cetirizine 10 mg or equivalent dose of another H1 blocker). For the premedication, paracetamol/acetaminophen is preferred over ibuprofen.

At the discretion of the Investigator, with input from Ultragenyx and the DMC, and based on the emerging safety profile of UX053, additional premedications and rescue medications may be used to reduce the risk and severity of immune reactions in RD cohorts. Premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications in patients with GSD III.

If a subject develops an IRR that meets diagnostic criteria for anaphylaxis (Section 8.3.3), epinephrine should be administered as initial rescue medication. For all other hypersensitivity reactions ≥ Grade 2 in severity suspected to be due to immune activation, dexamethasone should be the initial rescue medication. The Medical Monitor must be notified if any rescue medication is used.

If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor.

Section 8.3.2 provides subject-level redosing criteria, stopping criteria, and additional instructions on how to proceed in the event of an IRR.

Subjects will have cardiorespiratory monitoring throughout the infusions and will stay overnight for visits in which they receive study drug and be observed in an inpatient or observational unit for 24 hours after the end of each infusion. Prior to discharge, all the following criteria must be met:

- All scheduled study assessments have been completed.
- No evidence of vital sign instability or clinical instability in the opinion of the investigator.
- Any signs or symptoms of potential IRRs are resolving or resolved.

Guidance on signs and symptoms that subjects should be vigilant for upon discharge and how to proceed in the event that these signs and symptoms manifest are provided in Section 9.1.4.

In each SAD cohort, subjects will be dosed sequentially with review of safety laboratory data from the 24-hour time point prior to dosing of the next subject. Within SAD cohorts, dosing of individual subjects will occur at a minimum interval of 72 hours. For example, the second subject in a cohort receives their first dose after a minimum of 72 hours after the first subject in the same cohort received their first dose. Subjects within a given RD cohort can be dosed simultaneously. After the first dose, DB-RD subjects maintain a Q2W dosing regimen and OL-RD subjects maintain a Q4W dosing regimen.

Subjects may receive COVD-19 vaccines during participation in the study. To facilitate discrimination of UX053 IRRs and adverse effects of vaccine administration, vaccines should not be administered within 48 hours after the most recent administration of UX053 and until safety labs obtained have been reviewed and the subject has been assessed for any possible hypersensitivity reaction to the most recent dose of UX053 (Section 9.1.6).

For the DB-RD cohort, the subjects, Investigators, and site staff will be blinded to the treatment. Due to the difference in the opacity of UX053 and placebo, an unblinded pharmacist will prepare the investigational product (IP) and an unblinded nurse will prepare the infusion before masking the infusion syringe and tubing with colored covers.

The full schedules of events for the SAD, OL-RD and DB-RD, cohorts are listed in Table 4, Table 5, and Table 6, respectively. Additional details regarding the timing of assessments before, during, and following treatment infusion are provided in Table 7 for vital signs and Table 8 for PK samples.

Although they are eligible, it is expected that few patients with GSD IIIb, IIIc, or IIId will enroll. Subjects with GSD IIIb/c/d will participate in all assessments, including muscle imaging and function tests, and will be included in all safety analyses. Sensitivity analyses for cardiac and skeletal muscle endpoints may exclude patients with GSD IIIb/c/d as described in the SAP.

Future protocol amendments may occur to include pediatric subjects with GSD III or the addition of an open-label extension for all subjects after an interim review of safety, PK, and PD data.

7.1.1. Number of Sites

Approximately 10 sites globally will participate.

7.1.2. Number of Subjects

Approximately 18 adult subjects are planned to be enrolled. Additional subjects may be enrolled to further characterize safety.

7.1.3. Rationale for Study Design

The purpose of this study is to determine the safety, PK, PD, and preliminary efficacy of UX053 in adults with GSD III. The nutrition optimization and stabilization according to nutrition guidelines specific to GSD III aim to minimize confounding of safety and PD/efficacy data by dietary interventions. The duration of the nutrition optimization and stabilization period for the OL-RD cohorts is shorter than the same period for the DB-RD cohorts because nutrition data is collected and reviewed for OL-RD subjects during participation in the SAD cohorts. Screening includes assessments of the disease state and potential biomarkers that will inform the design of subsequent studies.

The wide range of screening assessments are designed to improve understanding of measurable pathology in adults with GSD III. Continued evaluation of these assessments in the RD cohorts will help to determine the preliminary efficacy of UX053. Post-treatment assessments for some procedures (eg, liver magnetic resonance imaging [MRI], liver magnetic resonance spectroscopy [MRS], and Fibroscan or liver ultrasound) are not performed during the SAD Period to minimize burden on sites and subjects.

The DB-RD cohorts are double-blind to prevent conscious or unconscious bias in safety and efficacy data collection. The SAD cohorts are open label, as RD better reflects dosing in a real-world setting and provides a more relevant safety profile; therefore, blinding to avoid bias is more critical for DB-RD cohorts. In an effort to further understand the safety, PK, and optimal dosing regimen of UX053, subjects in the SAD cohorts can enroll in OL-RD cohorts; these OL-RD cohorts allow for the investigation of a Q4W dosing regimen in previously exposed subjects.

Subject-level dose-reductions are designed to minimize risk to subjects and determine the optimal dose for subsequent cohorts and future studies. After data from Cohort 1S are reviewed, and with each subsequent data review, the ensuing cohort dose levels may be modified. After establishing safety in Cohort 2S, the RD cohorts will characterize the safety of RD of UX053.

To minimize reporting bias for safety and PD/efficacy assessments, placebo was chosen as the appropriate control for the DB-RD cohorts. Data from this FIH study will provide the basis for the initial safety profile of UX053. Enrolled subjects are expected to have signs and symptoms of GSD III, AEs related to the GSD III, and numerous laboratory abnormalities. Blinded treatment allocation reduces bias in reporting of AEs; the placebo control is helpful to determine whether UX053 exacerbates the underlying disease, or whether the events represent disease progression. Minimizing bias in the safety and PD data from this study is critical to inform the clinical development of UX053. An unbiased assessment of PD endpoints will allow for the identification and prioritization of clinically relevant endpoints in subsequent phases of clinical development.

There are no approved treatments for GSD III. All study participants will receive standard of care in addition to placebo or UX053. In fact, standard of care nutrition management guidelines have been incorporated into the protocol to ensure that standard of care will be followed (Section 7.1). The use of a placebo control will not impact standard of care. Once chronic toxicity data are available, a future protocol amendment is planned to allow all subjects, including those previously treated with placebo, to receive long-term open-label treatment with UX053.

7.1.4. Dose Rationale

The planned doses of UX053 (0.05, 0.10, and 0.20 mg/kg) aim to establish a safe dose of UX053 in adults with GSD III, as well as provide a preliminary PK and PD profile of UX053. These doses were chosen based on single-dose and repeat-dose pharmacology studies in mouse and dog models of GSD III, and RD toxicology and toxicokinetic studies in rats, cynomolgus monkeys, and Beagle dogs. The NOAEL in repeat-dose studies with 5 total doses of UX053 administered Q2W was CCI mg/kg in dogs, CCI mg/kg in rats, and CCI mg/kg in monkeys. The maximum tolerated dose (MTD) was CCI mg/kg in rats and CCI mg/kg in monkeys. The MTD in dogs is not known since higher doses were not evaluated with Q2W administration. The MTD in dogs with weekly dosing of UX053 is CCI mg/kg, but this short interval between doses is believed to promote an accumulation of toxicity that does not occur with Q2W dosing. Hence, the MTD in dogs may be greater than CCI mg/kg when UX053 is administered Q2W, but remains undetermined. CCI [redacted] Taken together, these PK and drug metabolism properties of the drug support 1:1 weight-based scaling from nonclinical species to humans, which is an approach endorsed by a cross-industry consortium of experts in oligonucleotide therapies, the Oligonucleotide Safety Working Group (Kornbrust, 2019; Marlowe et al., 2017). The FIH starting dose of 0.05 mg/kg is CCI less than the NOAEL in the most sensitive nonclinical species, the dog. The highest potential FIH dose of 0.30 mg/kg is CCI less than the dog NOAEL. Additional details regarding the use of weight-based scaling versus body-surface area scaling, as well as summaries of nonclinical studies with UX053 are available in the IB.

Sequential dosing with an interval of 72 hours between subjects' first dose within a cohort and a 2-week minimum review of data between cohorts was chosen to allow enough time to detect any potential TEAEs, including potential immune responses. In nonclinical studies, toxicities generally occurred soon after dosing and promptly resolved. The DB-RD cohorts will aim to achieve a steady state with Q2W dosing. The OL-RD cohorts will aim to evaluate a Q4W dosing regimen. Subjects will receive premedication prior to the treatment infusion to prevent hypersensitivity reactions, in line with previously established programs evaluating RNA therapeutics.

Subjects will receive premedication at least 1 hour prior to infusion. Study drug will be administered as an IV infusion over at least 4 hours, with a slower rate of infusion for the first hour to minimize the risk of anaphylactoid-type or hypersensitivity reactions (which may include IRRs). If a subject develops an IRR that meets diagnostic criteria for anaphylaxis (Section 8.3.3) epinephrine should be administered as initial rescue medication. For all other hypersensitivity reactions. ≥ Grade 2 in severity suspected to be due to immune activation, dexamethasone should be the initial rescue medication (Section 9.5). The Medical Monitor must be notified if any rescue medication is used. If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor. Section 8.3.2 provides subject-level redosing criteria, stopping criteria, and additional instructions on how to proceed in the event of an IRR. A similar premedication regimen that includes dexamethasone has proven effective for patisiran, a siRNA LNP treatment approved by the FDA in 2018 for the treatment of polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults (ONPATTRO).

7.1.5. Changes to the Protocol Due to Coronavirus Disease 2019 (COVID-19)

Changes to the protocol or Investigational Plan to minimize or eliminate immediate hazards or to protect the life and well-being of research subjects and/or study staff may be implemented without IRB/IEC approval or before filing an amendment to the competent authority but are required to be reported afterward. Investigators should contact Ultragenyx or Designee to determine an appropriate course of action, which may include but is not limited to remote visits, home health visits, and out of window visits or assessments. Any changes to the protocol or investigational plan must be documented. The Investigator will work with Ultragenyx or Designee and IRB/IEC to prioritize reporting of protocol deviations that impact safety of trial subjects.

7.2. Duration

7.2.1. Duration of Subject Participation

SAD Cohorts

Screening is approximately 2 weeks. After screening and dosing, subjects will be monitored for 90 days after dosing.

OL-RD Cohorts

Subjects who have completed participation in a SAD cohort, consisting of screening and a 90-day Follow-up Period, may enter an OL-RD cohort. These subjects are rescreened for participation in the OL-RD cohorts, which is expected to occur over the course of approximately 3 weeks prior to redosing; eligibility for additional dosing in the OL-RD cohorts can be combined with assessments at the SAD Day 90 Visit. After rescreening, subjects will receive a total of 4 additional doses of UX053, with doses administered Q4W, and will be monitored for 36 weeks (Week 48 Visit) after their last dose of UX053.

DB-RD Cohorts

Screening is approximately 8 to 12 weeks. Due to the staggered nature of the cohorts, subjects may remain in nutrition stabilization during screening for ≥ 6 weeks. After screening, subjects will receive a total of 5 doses of UX053, with doses administered Q2W, and will be monitored for 40 weeks (Week 48 Visit) after the last dose of study drug.

7.2.2. End of Study Definition

The end of the study is defined as the date of the last protocol-specified visit (including safety follow-up telephone calls in the event of early termination [ET]) for the last subject in the study.

8. POPULATION

Prospective protocol deviations for recruitment and eligibility criteria (ie, protocol waivers or exemptions) are not permitted.

8.1. Inclusion Criteria

Each subject must meet the inclusion criteria listed below at screening (ISV for DB-RD cohorts) or rescreening (OL-RD cohorts) and baseline (Day 0 for SAD cohorts or Week 0 in all RD cohorts), as applicable, to be enrolled in the study:

1. Confirmed diagnosis of GSD III (all subtypes) based on pathogenic mutations in the *AGL* gene on both alleles or GDE deficiency based on biopsy of liver, muscle, or fibroblasts
2. History of any of the following:
    a. Severe hypoglycemia, defined as neuroglycopenia (eg, altered mental status, seizure, dizziness, slurred speech, blurry vision, abnormal behavior, perioral paresthesia, requiring intervention by a caregiver) or blood glucose < 54 mg/dL (3 mmol/L) within the last year
    b. ≥ 2 incidents of symptomatic hypoglycemia (defined as blood glucose < 70 mg/dL [3.9 mmol/L] if measured at the time of symptoms) within the last year, despite nutrition management
    c. Ongoing liver injury, defined as alanine aminotransferase (ALT) > 2.5x the upper limit of normal (ULN) within the last year
3. For treatment-naïve subjects (SAD and DB-RD cohorts), ALT ≤ 5x the upper limit of normal (ULN) during the 3 months prior to the Baseline Visit.
4. Males or females ≥ 18 years of age
5. For subjects enrolling into DB-RD cohorts, willing and able to demonstrate nutrition stability based on nutrition guidelines for adults with GSD III (Table 2)
6. Willing and able to provide access to medical records surrounding medical treatment that occurred prior to enrollment and during the study
7. Willing and able to provide written informed consent, or in the case of adult subjects with cognitive limitation, provide written assent (if required) and written informed consent by a legally authorized representative after the nature of the study has been explained and prior to any test procedures or assessments
8. Females of childbearing potential must have a negative pregnancy test at screening (and rescreening, as applicable) and be willing to have additional pregnancy tests during the study. Subjects of child-bearing potential or males who are sexually active with partners of child-bearing potential must consent to use a highly effective contraceptive method, as described in Appendix 4, from the Period following the signing of the informed consent through 30 days after last dose of study drug
9. For subjects rescreening into OL-RD cohorts after treatment with UX053 in a SAD cohort, subjects must meet the following criteria:

a. If a significant rise in ALT occurs after the prior dose, ALT should show a decreasing trend toward the subject's baseline value
b. Total bilirubin is within normal limits
c. Platelets are within normal limits
d. International normalized ratio (INR) is within normal limits

8.2. Exclusion Criteria

Each subject must not meet the exclusion criteria listed below at screening (ISV for DB-RD cohorts) and baseline (Day 0 for SAD cohorts or Week 0 in all RD cohorts), as applicable, to be enrolled in the study:

1. History of liver transplant, including hepatocyte cell therapy/transplant, or active listing for liver transplant
2. History of cirrhosis, or presence of any of the following:
   a. Total bilirubin ≥ 1.3 mg/dL and INR ≥ 1.3
   b. Evidence of portal hypertension, including, but not limited to the following symptoms splenomegaly, ascites, thrombocytopenia, esophageal varices, or history of hepatic encephalopathy
   c. Model for End Stage Liver Disease (MELD) score > 12
3. Current Hepatitis B or C infection or history of chronic Hepatitis B or C infection
4. Severe renal impairment defined as a glomerular filtration rate (GFR) ≤ 29 mL/min (Levey et al., 2005).
5. Any prior history of hepatocellular carcinoma or presence of liver adenoma > 5 cm at the longest diameter or > 3 cm and ≤ 5 cm in size that has an annual growth rate of ≥ 0.5 cm per year
6. Current or history of malignancies in the 3 years prior to the Screening Visit (ISV for RD)
7. Hospitalizations related to GSD III disease between the Screening (ISV for RD) and Baseline Visit
8. Known history of human immunodeficiency virus infection
9. Presence or history of any hypersensitivity reactions requiring medical evaluation and management (including injection/IRRs, such as lymphadenopathy) to UX053, its excipients, or any drug products that contain polysorbate or PEG. This may include vaccines that contain PEG or polysorbate
10. Significant cardiac disease, including heart failure with New York Heart Association (NYHA) Function Capacity III or IV or Objective Assessment C or D, unstable angina, or ejection fraction (EF) < 35%, or uncontrolled arrhythmia or resistant hypertension (Carey et al., 2018). Mild cardiomyopathy and left ventricular hypertrophy (LVH) are allowed
11. Presence or history of any co-morbid condition or abnormal labs that, in the view of the Investigator, places the subject's safety at risk; places the subject at high risk of poor

treatment compliance or not completing the study; or would significantly affect the interpretation of study results

12. Poorly controlled diabetes, defined as the presence of any of the following:
    a. Hemoglobin A1C > 8% (Qaseem et al., 2018)
    b. History of diabetic nephropathy, neuropathy, or retinopathy
    c. History of diabetic ketoacidosis during the past year
13. Poorly controlled hypothyroidism, based on the judgement of the Investigator or Ultragenyx, whichever is most conservative
14. History of chronic coagulopathy, thrombophilia, or disorder of complement activation
15. Use of concomitant medications that alter prothrombin time/international normalized ratio (PT/INR), including warfarin and direct oral anticoagulants (eg, rivaroxaban, apixaban, and edoxaban). Patients who receive medications that affect platelet function, such as aspirin or clopidogrel, are allowed unless they have comorbidities that in the judgment of the Investigator place them at undue risk to participate in the study.
16. Current treatment with long-term immunosuppressive medications. This includes subjects with autoimmune conditions managed with immunosuppressive medications and solid organ transplant recipients.
17. Active tuberculosis requiring treatment in the past 3 years
18. Symptomatic COVID-19 infection
19. History of active alcohol and/or drug abuse that in the Investigator's assessment would impair the subject's ability to comply with the protocol
20. Receipt of only 1 of 2 planned doses of a COVID-19 vaccine. Subjects who have not received a COVID-19 vaccine, and those who have completed COVID-19 vaccination are eligible.
21. Planned surgery, including dental surgeries, during the study
22. Pregnant or breastfeeding or planning to become pregnant (self or partner) at any time during the study
23. Females of childbearing potential with hepatocellular adenoma who are unwilling to use nonhormonal contraception
24. Use of any IP or investigational medical device within 30 days or for IP within 5 half-lives, whichever is longer, prior to screening (or rescreening, as applicable), or requirement for any investigational agent prior to completion of all scheduled study assessments
25. For subjects rescreening into OL-RD cohorts, any of the following after treatment with UX053 in a SAD cohort:
    a. New or worsening symptoms of liver disease (including new or worsening hepatomegaly) along with any increase in transaminase levels
    b. Receipt of any blood product administration (eg, packed red blood cells, platelet, FFP) for management of consumptive coagulopathy

c. An ALT level that is ≥ 8x ULN and > 2x the subject's baseline value in the absence of an alternative explanation

8.3. Subject Discontinuation and Stopping Rule Criteria

8.3.1. Discontinuation of Intervention, Subject, or Study

Subjects have the right to discontinue study drug or withdraw from the study at any time for any reason. Subjects will only be removed from the study due to withdrawal of consent. The Investigator and Ultragenyx also have the right to discontinue a subject's treatment to protect subject's safety (Section 8.3.2), but subjects will remain in the study for safety monitoring purposes, unless continued participation in the study poses a risk to the subject due to a concurrent medical condition or illness. Ultragenyx must be notified of all subject withdrawals as soon as possible. Ultragenyx also reserves the right to discontinue participation of an Investigator due to poor enrollment or noncompliance, as applicable.

Subjects who are discontinued from study drug treatment because of safety concerns are encouraged to remain in the study and to continue to complete study assessments to the extent possible. If a subject discontinues from the study prematurely, reasonable efforts should be made to perform the ET Visit procedures within 4 weeks of discontinuation. In the event a subject has an ET, all efforts will be made to monitor the subject through the end of the study. At minimum, a safety follow-up phone call will occur within the 4 weeks following the subject's last treatment.

If a subject is withdrawn from treatment due to safety reasons or if a subject has an ongoing SAE at the end of participation, the subject should be followed until the event resolves, the subject's condition has stabilized, or until a pre-defined outcome is reached.

8.3.2. Subject-level Redosing and Stopping Criteria

At any time after the first dose of study drug, if a marked increase in transaminases (ie, > 2x the subject's baseline value and 3x ULN) is observed, liver enzymes, bilirubin, and INR should be obtained again within 48 to 72 hours. Study drug should not be re-administered unless the following criteria are met:

- If a significant rise in ALT occurs after the prior dose, ALT should show a decreasing trend toward the subject's baseline value
- Total bilirubin is within normal limits
- Platelets are within normal limits
- INR is within normal limits

Additionally, subjects who meet any of the following criteria will not be redosed:

- Subjects who develop new or worsening symptoms of liver disease (including new or worsening hepatomegaly) along with any increase in transaminase levels
- Subjects who require any blood product administration (eg, packed red blood cells, platelet, fresh frozen plasma) for management of consumptive coagulopathy

- Subjects who develop an ALT level that is ≥ 8x ULN and > 2x the subject's baseline value in the absence of an alternative explanation

If a subject develops an IRR that meets diagnostic criteria for anaphylaxis (Table 11), epinephrine should be administered as initial rescue medication. For all other hypersensitivity reactions ≥ Grade 2 in severity suspected to be due to immune activation, dexamethasone should be the initial rescue medication. The Medical Monitor must be notified if any rescue medication is used.

If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor.

In the event of an IRR, action outlined in Table 10 should be implemented based on the level of severity. Ultragenyx will confer with the DMC whenever subject- or study-level stopping criteria are potentially met.

Table 10: Action in the Event of an Infusion-Related Reaction

IRR CTCAE Severity	Action
Grade 1 (Mild)	The infusion may be slowed or interrupted temporarily and resumed at the original speed
Grade 2 (Moderate)	The infusion should be interrupted. The post-infusion complement and cytokine labs should be obtained within 1 hour of the start of the reaction. The subject should be closely monitored, and supportive care should be provided. Dexamethasone 10 mg IV (or equivalent) should be administered. Additional treatment, including a complement inhibitor (such as eculizumab) may be considered. The infusion may be restarted at half the rate after symptomatic treatment and resolution of the reaction. If the subject has IRR following resumption at the lower rate, stop the infusion.
Grade ≥ 3 (Severe, Life-threatening, Death)	The infusion should immediately be terminated. The post-infusion complement and cytokine labs will be obtained within 1 hour of the start of the reaction. The subject should be closely monitored, and supportive care should be provided. Dexamethasone 10 mg IV (or equivalent) should be administered. Additional treatment, including a complement inhibitor (such as eculizumab) may be considered. The subject will not be redosed.

CTCAE, Common Terminology Criteria for Adverse Events; IRR, infusion-related reaction; IV, intravenous.

8.3.3. Study Stopping Criteria

Study enrollment and any further administration of study drug for subjects already enrolled, will be paused and the DMC and regulatory authorities will be notified if, at any time during the study, any of the criteria listed below occur after UX053 administration:

- Death of a subject
- Any serious TEAE with a severity ≥ Grade 3 that is assessed as related to study drug or study procedures by the Investigator or Ultragenyx
- Any TEAE with a severity ≥ Grade 3 affecting the cardiopulmonary, renal, or neurological systems, regardless of relationship to study drug, pending a DMC safety review

- Anaphylaxis, as defined by the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network (NIAID FAAN) criteria (Table 11), pending a DMC safety review
- Increases in ALT or AST > 2x from Baseline and > 3x the ULN, accompanied by total bilirubin > 2x ULN or INR > 1.5, without findings of cholestasis (defined as serum ALP activity < 2x ULN) and in the absence of a plausible alternate explanation
- Increases in serum concentrations of ALT or AST > 2x from Baseline and > 3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia (> 5% of total leukocytes)
- 3 subjects develop platelet counts < 50,000 per mm^3 (or thrombocytopenia ≥ Grade 3 in severity)
- 3 subjects develop INR > 1.5 (or INR increase ≥ Grade 2 in severity)

In addition to the above criteria, the DMC may at any time recommend dose reduction or discontinuation of dosing for an individual subject or for all subjects (Section 9.1.5). Regulatory authorities will be notified immediately if the study stopping criteria are met and study enrollment or dosing is halted.

Table 11: Clinical Criteria for Diagnosing Anaphylaxis

Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:
1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula) *AND AT LEAST ONE OF THE FOLLOWING* a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure to a *likely allergen for that patient* (minutes to several hours): a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula) b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence) d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)
3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours): a. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

PEF, Peak expiratory flow; BP, blood pressure.
[a] Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg 1 [2 3 age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years.
Source: (Sampson et al., 2006)

8.3.4. Subject Replacement

Subjects will be replaced at Ultragenyx's discretion only if they have not received study drug. Subjects will not be replaced if they have received any study drug.

8.4. Informed Consent

Informed consent must be obtained from the patient before performing any study-related procedures.

In the event that a subject is rescreened (Section 8.5), a new informed consent form (ICF) must be signed.

8.5. Screening Requirements

All screening evaluations specified in Table 4, Table 5, and Table 6 must be completed and reviewed to confirm eligibility. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

At the Screening Visit for SAD cohorts and ISV for DB-RD cohorts, informed consent will be obtained, and general medical information (ie, demographics, general medical history, and GSD III specific medical history), a complete physical exam, vital signs, anthropometrics, concomitant medications, and dietary information will be collected. Subjects will also be screened for hepatitis B and C. Several laboratory assessments, as outlined in Table 4, Table 5, and Table 6, will be collected during screening.

Subjects in the SAD cohorts who complete the 90-day Follow-up Period can elect to enter OL-RD cohorts. These subjects will complete an abbreviated rescreening for participation in the OL-RD cohorts, occurring over the course of at least 3 weeks prior to redosing; eligibility for additional dosing in the OL-RD cohorts (ie, rescreening) can be combined with assessments at the SAD Day 90 Visit. Informed consent for participation in the OL-RD cohorts must be signed prior to rescreening.

Rescreening for subjects who previously failed screening is permissible on a case-by-case basis. Each case should be discussed with the Medical Monitor. If a subject is rescreened for the DB-RD cohorts, the subject will still be required to complete up to 6 weeks of nutrition stabilization during screening. Subjects who undergo rescreening for DB-RD cohorts may not need to repeat the imaging assessments.

9. INTERVENTION(S)

An overview of all interventions, including the IP, premedications, and rescue medications are provided in Table 12.

Table 12: Study Investigational Product, Premedication, and Rescue Medication

	Intervention[a]					
	UX053	**Placebo**	**Paracetamol/ acetaminophen or ibuprofen[b]**	**H1 blocker; cetirizine or equivalent**	**H2 blocker; famotidine or equivalent**	**Dexamethasone or equivalent[c]**
Dose Formulation	Ampule	Ampule	Tablet	Tablet	Tablet	Ampule
Unit Dose Strength(s) / Dosage Level(s)	0.05, 0.10, and 0.20, mg/kg single dose during in SAD cohorts and Q2W or Q4W in RD cohorts[d]	-	500 mg (paracetamol /acetaminophen); 400-800 mg (ibuprofen)	10 mg for cetirizine	20 mg for famotidine	10 mg for IV dexamethasone
Route of Administration	IV infusion	IV infusion	Oral	Oral	Oral	IV infusion
Use	Experimental	Placebo	Premedication[e]	Premedication[e]	Premedication[e]	Rescue medication, or premedication for subsequent infusions if used as rescue medication previously

[a] Regardless of assigned treatment (UX053 or placebo), all subjects in DB-RD cohorts will also be asked to maintain a diet based on expert recommendations for adults with GSD III throughout the study (Table 2).

[b] Paracetamol/acetaminophen is preferred over ibuprofen.

[c] Dexamethasone should be the first-in-line rescue medication; alternative or additional rescue medications are allowed, if necessary, at the discretion of the Investigator. The Medical Monitor must be notified if any rescue medication is used.

[d] Additional cohort(s) at dose levels between 0.05 mg/kg to 0.30 mg/kg may also be included pending review of safety data.

[e] Premedications will be administered to all subjects, including subjects receiving placebo. At the discretion of the Investigator, with input from Ultragenyx and the DMC, and based on the emerging safety profile of UX053, additional premedications and rescue medications may be used to reduce the risk and severity of immune reactions in RD cohorts. Premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications in patients with GSD III.

DB, double-blind; DMC, Data Monitoring Committee; GSD, Glycogen Storage Disease; IV, intravenous; Q2W, every 2 weeks; Q4W, every 4 weeks; RD, Repeat Dose; SAD, Single Ascending Dose.

9.1. UX053

UX053 consists of an mRNA encoding full-length, human GDE encapsulated in an LNP. CCI [redacted] Both UX053 and placebo will be diluted prior administration such that the concentration of components, including sucrose, is identical. The final concentration (for both UX053 and placebo) will be CCI [redacted] Additional information on physical and chemical properties of UX053 are provided in the IB.

UX053 is supplied in a frozen sterile solution in a vial. UX053 should be stored in a freezer, set to maintain CCI [redacted] ° C, protected from light. UX053 is manufactured, packaged and labelled according to current Good Manufacturing Practices (GMP).

9.1.1. Premedications

The study site will supply premedications that will be obtained locally. Subjects will receive premedication at least 1 hour prior to the infusion, consisting of oral paracetamol/acetaminophen (500 mg) or ibuprofen (400 to 800 mg), an H2 blocker (eg, famotidine 20 mg or equivalent dose of another H2 blocker), and an H1 blocker (eg, cetirizine 10 mg or equivalent dose of another H1 blocker). For the premedication, paracetamol/acetaminophen is preferred over ibuprofen. At the discretion of the Investigator, with input from Ultragenyx and the DMC, and based on the emerging safety profile of UX053, additional premedications and rescue medications may be used to reduce the risk and severity of immune reactions in RD cohorts. Premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications in patients with GSD III. If a subject develops an IRR that meets diagnostic criteria for anaphylaxis (Section 8.3.3), should be administered as initial rescue medication. For all other hypersensitivity reactions ≥ Grade 2 in severity suspected to be due to immune activation, dexamethasone should be the initial rescue medication (Section 9.5). The Medical Monitor must be notified if any rescue medication is used. If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor. Section 8.3.2 provides subject-level redosing criteria, stopping criteria, and additional instructions on how to proceed in the event of an IRR.

9.1.2. Preparation and Administration

For detailed preparation and administration information, refer to the Pharmacy Manual. Prior to each infusion, the amount of study drug will be determined based on subject weight (in kg). The volume of study drug calculated to deliver the correct dose will be withdrawn from the vial. Study drug will be diluted and transferred to an infusion bag as a final dose. Undiluted UX053 must never be infused.

Study drug is administered as an IV infusion over the course of at least 4 hours, with a slower rate of infusion for the first hour to minimize the risk of hypersensitivity or anaphylactoid-type reactions. The infusion rate schedule may be slowed to minimize the risk of IRRs in individual

subjects. Study drug is administered once in the SAD cohorts and either Q2W or Q4W in the RD cohorts.

9.1.3. Clinical Observation and Supportive Care

Subjects will stay overnight onsite on for all dosing visits for safety and PK assessments. If a subject develops an IRR that meets diagnostic criteria for anaphylaxis (Section 8.3.3), epinephrine should be administered as initial rescue medication. For all other hypersensitivity reactions ≥ Grade 2 in severity suspected to be due to immune activation, dexamethasone should be the initial rescue medication; alternative or additional rescue medications are allowed, if necessary, at the discretion of the Investigator. The Medical Monitor must be notified if any rescue medication is used. If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor. Section 8.3.2 provides subject-level redosing criteria, stopping criteria, and additional instructions on how to proceed in the event of an IRR. Table 7 and Table 8 show the schedule for vital signs and PK assessments, respectively, occurring before, during, and after study drug infusion.

9.1.4. Guidance on Communication and Seeking Immediate Medical Attention

Subjects should inform their primary care provider of their participation in the UX053-CL101 clinical trial. Communication between the subject's primary care provider and study Investigators is strongly encouraged, and study Investigators should share visit summaries and hospital discharge summaries with the subject's primary care provider. Subjects should contact their primary care provider for routine care outside the management of GSD III.

At any time during the study, subjects should contact the Investigator if they notice any changes in their health or any other symptoms that cause the subject distress. Based on the clinical assessment and judgement of the study Investigator, the subject may be referred to their primary care physician, to seek treatment in an emergency room, or to contact local emergency services.

At any time during the study, subjects should seek immediate medical attention and notify the Investigator if they develop any of the following signs or symptoms:

- Signs of a severe hypersensitivity reaction, such as generalized urticaria, angioedema, respiratory distress, or anaphylaxis
- Jaundice
- Unusual bleeding or bruising
- Altered mental status, seizure, syncope, dizziness, or presyncope
- Severe chest or abdominal pain
- Blood sugar below 54 mg/dL (3 mmol/L) accompanied by symptoms of neuroglycopenia (dizziness, weakness, drowsiness, confusion, or altered mental status)

9.1.5. Dose Modification

If a subject develops a TEAE/serious TEAE ≥ Grade 3 that is considered by the Investigator to be related to study drug or an intolerable TEAE in the RD cohorts, subject-level dose reductions are allowed in consultation with the Medical Monitor, as outlined in the guidance provided in the Pharmacy Manual. At any time, the DMC may recommend discontinuation of dosing for an individual subject or for all subjects. At any time, Ultragenyx may modify or discontinue dosing for an individual subject or for all subjects.

Dose levels for each cohort, in either the SAD or RD cohorts, may be reduced and/or higher dose cohorts may not be initiated, depending on safety and PD findings from prior cohorts, as determined by Ultragenyx (with input from the DMC for the RD cohorts).

9.1.6. Coronavirus Disease 2019 (COVID-19) Vaccination

Both UX053 and the mRNA-LNP vaccines for SARS-CoV-2 contain some of the same excipients, which are the LNP structural lipid components, namely CCI Currently available mRNA-LNP COVID-19 vaccines also contain either the same CCI or a similar CCI as UX053. Patients who have experienced a severe hypersensitivity reaction in response to an mRNA-LNP vaccine are excluded from this study due to potential cross-reactivity of antibodies to UX053, such as CCI antibodies (Section 8.2).

While COVID-19 vaccination is allowed during study participation, COVID-19 vaccines should not be administered within 48 hours after the most recent administration of UX053 and until safety labs obtained have been reviewed and the subject has been assessed for any possible hypersensitivity reaction to the most recent dose of UX053. The timing of COVID-19 vaccination doses will be captured in the case report form (CRF), as these would inform the interpretation of AEs.

If a subject has an immediate or severe hypersensitivity reaction to UX053, consultation with an allergist/immunologist is recommended prior to administration of an COVID-19 vaccine due to potential antibody cross-reactivity CCI (Banerji et al., 2020). It is not known whether treatment with UX053 increases the likelihood of developing a severe hypersensitivity reaction to an mRNA-LNP vaccine. For additional information on potential interventions in the event of toxicity, please refer to the IB.

9.2. Reference Therapy

A placebo consisting of the UX053 formulation buffer (without UX053) will be supplied as a sterile solution in a vial. Placebo should be stored in a refrigerator, set to 2 to 8° C, protected from light. The placebo consists of the same components as the formulation buffer for UX053. Placebo is manufactured, packaged and labelled according to current GMPs. Both placebo and UX053 will be diluted prior to administration such that the concentration of components, including sucrose, is identical. The final concentration (for both placebo and UX053) will be CCI

9.2.1. Preparation and Administration

Preparation, administration, observation, and supportive care procedures for placebo are similar to those described for UX053 (Section 9.1.2). For detailed preparation and administration information, refer to the Pharmacy Manual.

9.3. Nutrition Management

Subjects in the OL-RD cohorts will be assessed to determine if their diet meets nutritional guidelines based on expert recommendations for adults with GSD III (Table 2). Once optimized, subjects in the OL-RD cohort are required to demonstrate nutrition stability for 2 weeks prior to their Baseline/Week 0 Visit (Section 7.1). Subjects are instructed not to make any substantial changes to their diet throughout the remainder of the study. If an OL-RD subject is unable to achieve an optimized diet, they are still eligible to proceed to the OL-RD Baseline/Week 0 Visit. The reason for the inability to achieve an optimized diet should be documented and the subject must agree not to make any substantial changes to their diet throughout the remainder of the study.

Regardless of assigned treatment (UX053 or Placebo), subjects in the DB-RD cohorts will be assessed to determine if the subject's diet meets nutritional guidelines based on expert recommendations for adults with GSD III (Table 2). Once the diet is optimized, subjects are required to demonstrate maintenance of this diet for at least 6 weeks prior to their DB-RD Baseline Visit/Week 0 Visit (Section 7.1). Subjects should not make any substantial changes to their diet throughout the remainder of the study.

Nutrition guidance and monitoring for this study was established with available health authority guidance, and greater detail is available in the Study Reference Manual.

9.4. Prior and Concomitant Therapy

Subjects may receive concomitant medications as required, with the exception of those identified in Section 9.6 as prohibited.

The study site should indicate to laboratories if the subject is using any high dose biotin supplements (found in multivitamins, biotin supplements, and some supplements to promote hair, skin, and nail growth) to potential risk of assay interference (eg, troponin) (Section 10.1.5) (FDA, 2019a).

9.5. Rescue Medications

The study site will supply rescue medication that will be obtained locally. Dexamethasone (or equivalent) may be used as a rescue medication for hypersensitivity reactions (which may include IRRs) as determined by the Investigator. Alternative or additional rescue medications are allowed, if necessary, at the discretion of the Investigator. Premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications in patients with GSD III.

- Dexamethasone should generally be the initial rescue medication for infusion-related reactions. However, if diagnostic criteria for anaphylaxis are met (Table 11), epinephrine should be provided immediately.

- In the event of severe consumptive coagulopathy, treatment with blood products such as fresh frozen plasma (FFP)/cryoprecipitate and/or platelets may be considered.
- If profound complement activation is suspected, a complement inhibitor such as eculizumab may be considered.

For additional guidance, please refer to the Investigator Brochure.

The Medical Monitor must be notified if any rescue medication is used. If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for that subject's subsequent infusions after discussion with the Medical Monitor.

The use of rescue medications is allowable at any time during the study.

Section 8.3.2 provides subject-level redosing criteria, stopping criteria, and additional instructions on how to proceed in the event of an IRR.

9.6. Restricted or Prohibited Medications, Devices, and Procedures

The following are specifically prohibited during study participation, as noted in the exclusion criteria (Section 8.2):

- Use of any IP or investigational medical device within 30 days or for IP within 5 half-lives, whichever is longer, prior to screening (or rescreening, as applicable), or requirement for any investigational agent prior to completion of all scheduled study assessments
- Planned surgery, including dental surgeries, during the study

If a subject is on a drug that is metabolized primarily by cytochrome P450 2B6 (CYP2B6), caution is advised as UX053 may result in decreased CYP2B6 activity and altered exposure of the drug. Additional details regarding this potential interaction and nonclinical studies with UX053 are available in the IB.

9.7. Treatment Compliance

Study drug will be administered at the study site via a single IV infusion by qualified personnel. The dose, start time, stop time, and volume of infusion will be recorded in the subject's CRF.

Nutrition management and compliance in the RD cohorts will be monitored by reviewing subjects' nutrition diary data at time points specified in Table 5 and Table 6 and comparing this information with nutrition guidelines for adults with GSD III (Table 2). Nutrition guidance and monitoring for this study was established with available health authority guidance, and greater detail is available in the Study Reference Manual.

9.8. Treatment Assignment and Blinding

9.8.1. Treatment Assignment

In the SAD and OL-RD cohorts, all subjects receive open-label UX053. Subjects in the DB-RD cohorts are randomized 3:1 to UX053 and Placebo, using an interactive response technology (IRT) system. Subjects in the OL-RD cohorts receive open-label UX053.

Each subject will be assigned a unique identification number.

9.8.2. Blinding

Blind conditions will be established so that Ultragenyx, the subject, and site personnel will not know the identity of a subject's treatment in the DB-RD cohorts. Randomization using an IRT system will ensure subjects in the DB-RD cohorts are blind to treatment. Due to the difference in opacities of the UX053 and the placebo, an unblinded pharmacist will prepare the study drug and an unblinded nurse will prepare the infusion before masking the infusion syringe and tubing with colored covers. Prespecified employees from Ultragenyx who do not have any contact with the investigative sites may also be unblinded as specified in the Blinding Plan.

All imaging assessments will be sent to central readers for evaluation. Readers will be blinded to the subject's treatment.

Treatment assignment for an individual subject should be unblinded by the Investigator only in an emergency, and only if when knowledge of the treatment assignment is urgently needed for the clinical management or welfare of the subject. Unblinding at the study site for any other reason will be considered a protocol deviation.

The Investigator is strongly encouraged to contact Ultragenyx before unblinding any subject's treatment assignment, but priority should be given to the safety of the subject.

10. ASSESSMENTS

Assessments and associated timing are provided in Table 4, Table 5, and Table 6 for SAD , OL-RD, and DB-RD cohorts, respectively. Protocol waivers or exemptions are not allowed.

10.1. Safety Assessments

Safety will be evaluated by the incidence and severity of TEAEs and by changes from study baseline to scheduled time points in vital signs, weight, physical examination, clinical laboratory evaluations, and ECGs. In addition, safety will be evaluated by the development of ADA to CCI and GDE, concomitant medications, and monitoring pregnancies (or pregnancy of partner, if occurs or applicable). ADA will be assessed before the first dose of study drug and throughout the study.

Safety definitions and instructions for assessing severity and causality are presented in Appendix 1.

Refer to Appendix 2 for instructions regarding AE collection, including collection period timeframes and timelines for AEs, SAEs, pregnancies, other safety information, and any urgent safety measures taken to protect the safety or welfare of subjects.

10.1.1. Coronavirus Disease 2019 (COVID-19) Testing

COVID-19 testing by real time – polymerase chain reaction (RT-PCR) will be performed at time points specified in Table 4 and Table 6.

10.1.2. Vital Signs

Vital signs to be measured include seated systolic blood pressure and diastolic blood pressure, heart rate, respiration rate, temperature, and oxygen saturation. Vital signs are assessed at study visits specified provided in Table 4, Table 5, and Table 6. The schedule of vital sign assessments during dosing visits is provided in Table 7. Any new, clinically significant vital sign abnormalities should be captured as AEs.

10.1.3. Electrocardiogram

Single 12-lead ECG will be obtained using an ECG machine that automatically calculates the heart rate and measures RR, PR, QRS, QT, and QTc intervals. ECGs are performed at time points specified in Table 4, Table 5, and Table 6. While conduction intervals provided by the ECG machine may be used, final interpretation of ECGs, including any rhythm abnormalities, must be performed by a qualified local physician. Any new, clinically significant findings identified by ECG interpretation should be captured as AEs.

10.1.4. Physical Examinations

Complete and targeted physical exams are performed at time points specified in Table 4, Table 5, and Table 6. For visits in which subjects are receiving study drug, physical exams should occur prior to dosing.

A complete physical examination will include, at a minimum, assessments of the dermatologic; head, eyes, ears, nose, and throat (HEENT); respiratory; cardiovascular; gastrointestinal;

endocrine and metabolic; blood and lymphatic; musculoskeletal, psychiatric, and neurologic (including mental status, cranial nerves, motor system, reflexes, coordination and gait, and sensory system) systems.

A targeted physical examination will include, at a minimum, assessments of the respiratory, cardiovascular, and gastrointestinal systems.

Investigators should pay special attention to clinical signs related to previous serious illnesses. Any new, clinically significant findings from physical examinations should be captured as AEs.

10.1.5. Safety Laboratory Tests

Blood and urine samples will be collected for safety laboratory tests as specified in Table 4, Table 5, and Table 6. Fasting is not required. For visits in which subjects are receiving study drug, laboratory samples will be collected prior to dosing. All efforts should be made for urine collections to be from the first morning void. Samples will be processed by a central lab to ensure consistency across study sites.

In addition to chemistry, hematology, and urinalysis, additional assessments to monitor safety include activation of the complement pathway, coagulation factors, and interleukin-6. As noted in Table 4, Table 5, and Table 6, samples for complement pathway, coagulation factors, and interleukin-6 are collected pre- and post-infusion. Post-infusion blood samples should be collected in the opposite arm of the infusion to avoid laboratory artefacts. The full list of safety laboratory evaluations to be performed in this study are listed in Appendix 3. All laboratory samples listed in Appendix 3 will be processed by a central laboratory, except urine pregnancy.

The study site should indicate to laboratories if the subject is taking high dose biotin supplements (found in multivitamins, biotin supplements, and some supplements to promote hair, skin, and nail growth) to potential risk of assay interference (eg, troponin) (FDA, 2019a).

The Investigator (and/or Medical Monitor) may at his/her discretion perform additional laboratory tests for subject safety if a subject experiences an SAE or other AE that may warrant further follow up.

Close monitoring is required for subjects who develop elevations in ALT > 3x ULN and > 2x their baseline value, or elevation in ALT > 3x ULN with INR > 1.5. This includes repeating liver enzymes and INR within 48 hours and consideration of additional diagnostic tests such as liver ultrasound.

10.1.6. Anti-drug Antibodies

Blood will be collected to measure serum CCI [redacted] antibody level and anti-GDE antibody level using validated electrochemiluminescent methods at time points specified in Table 4, Table 5, and Table 6. For visits in which subjects are receiving study drug, blood will be collected prior to dosing.

10.1.7. Pregnancy Testing

Female subjects of childbearing potential will have urine pregnancy tests at visits indicated in Table 4, Table 5, and Table 6. For visits in which subjects are receiving study drug, results of

urine pregnancy testing should be reviewed prior to dosing. Females not of childbearing potential are not required to undergo pregnancy testing. Refer to Appendix 4 for contraception guidance.

Female subjects with a positive pregnancy test at the Screening Visit (ISV for DB-RD) will not be enrolled in the study.

Additional pregnancy tests will be performed at any visit in which pregnancy is in question. A serum pregnancy test will be performed in the event of a positive or equivocal urine pregnancy test result or can be performed if pregnancy test by urine is not feasible.

Refer to Appendix 2 for pregnancy reporting requirements.

10.1.8. Concomitant Medications

Medications (investigational, prescription, over the counter, vaccines, and herbal supplements) and nutrition supplements taken during screening will be reviewed and recorded at all screening visits (ISV through STV). Use of any concomitant medications during the study should be recorded on the CRF. All concomitant medications will be coded according to the World Health Organization (WHO) Drug Dictionary.

Site personnel should record date the medication was taken, the name of the medication, medication dosage, formulation, route of administration and the reason the medication was taken.

10.2. Pharmacokinetic Assessments

Serial blood and plasma samples will be collected to analyze for the concentrations of *AGL* mRNA and ATX95 at visits specified in Table 4, Table 5, and Table 6. *AGL* mRNA will be analyzed using a CCI [redacted]. ATX95 will be analyzed using a CCI [redacted] The schedule for PK assessments during dosing visits is provided in Table 8.

Pharmacokinetic parameters will be derived by non-compartmental analysis. PK parameters to be estimated may include, but not limited to, the following: time of maximum observed concentration (T_{max}), maximum concentration (C_{max}), area under the concentration-time curve from time 0 to the last measurable concentration (AUC_{last}), AUC from time 0 to infinity (AUC_{inf}), AUC from time 0 to end of dosing period (AUC_{tau}), accumulation ratio (calculated as AUC after repeat dose / AUC after a single dose; R_{AUC}), time of last measurable concentration (T_{last}), half-life ($T_{1/2}$), clearance (CL), and volume of distribution at steady state (V_{ss}).

Residual PK samples may be used for exploratory analysis to identify metabolites.

10.3. General Assessments

10.3.1. General Medical History and GSD III-specific Medical History

General medical history and GSD III-specific medical history will be collected during screening for the SAD and DB-RD cohorts (Table 4 and Table 6). General medical information includes subject demographics and a history of major medical illnesses, diagnoses, and surgeries. The GSD III-specific medical history will collect information on GSD III diagnostic history; current

and past disease management; GSD III-specific symptoms, conditions, and progression; and resource utilization, including physical/occupational therapy.

10.3.2. Demographics

Demographic information will be collected during screening for the SAD and DB-RD cohorts (Table 4 and Table 6).

10.3.3. Anthropometric Assessments

Height will be measured during screening for the SAD and DB-RD cohorts (Table 4 and Table 6). Weight will be assessed for all cohorts at time points specified in Table 4, Table 5, and Table 6. For visits in which subjects are receiving study drug, weight will be measured prior to dosing and used to calculate dose (Section 9.1.2). Additional details regarding anthropometric assessments are available in the Study Reference Manual.

10.4. Efficacy Assessments

10.4.1. Liver Assessments

10.4.1.1. Liver Magnetic Resonance Imaging

The volume of the liver will be determined using MRI and compared with normal values for age and sex. The liver MRI will also be analyzed to determine hepatic fat fraction. MRIs will be conducted at the study site; however, all results will be transmitted to a central radiologist for interpretation to ensure consistent assessment across study sites. Additional details regarding imaging sequences to be performed and instructions for transmitting MRI results are provided in the Imaging Manual.

Liver MRI will be performed at time points specified in Table 4, Table 5, and Table 6.

If feasible, glycogen nuclear overhauser enhancement (glycoNOE) imaging (Zhou et al., 2020) of the liver, may be collected at the time of the liver MRI.

10.4.1.2. Liver Magnetic Resonance Spectroscopy

When locally available, MRS will be used to quantitate glycogen in the liver. Scanning for liver MRS can be conducted immediately following the liver MRI, as both the MRI and MRS are conducted in an MRI machine. MRS will be conducted at the study site; however, all results will be transmitted to a central radiologist for interpretation and to ensure consistent assessments across study sites. Additional details regarding imaging sequences to be performed and instructions for transmitting MRS results are provided in the Imaging Manual.

Liver MRS will be performed at time points specified in Table 4, Table 5, and Table 6.

10.4.1.3. FibroScan or Liver Ultrasound Elastography

Transient elastography or the FibroScan System (Echosen; Paris, France) is a non-invasive, ultrasound-based technique to assess stiffness of soft tissue or, in the case of the liver, fibrosis and cirrhosis (Patel and Wilder, 2014; Castera et al., 2008). The technique allows for the measurement of velocity (m/s) of an elastic shear wave to provide a liver stiffness measurement

expressed in kilopascals (kPa), which correlates with fibrosis stage. Steatosis is measured using a controlled attenuation parameter score. In the event that FibroScan equipment is not available, liver ultrasound elastography will be performed.

FibroScan or liver ultrasound elastography will be performed at time points specified in Table 4, Table 5, and Table 6. FibroScan or liver ultrasound elastography will be conducted at the study site, and results will be sent to a central reader to ensure consistent assessments across study sites.

10.4.1.4. Prothrombin Time/International Normalized Ratio

Blood will be collected for the measurement of prothrombin time (PT) and to calculate PT/INR to aid in the assessment of liver health at time points specified in Table 4, Table 5, and Table 6. For visits in which subjects are receiving study drug, laboratory samples will be collected prior to dosing. Samples will be processed by a central lab to ensure consistency across study sites.

10.4.1.5. High Density Lipoprotein and Low Density Lipoprotein

Blood with be collected to measure serum high density lipoprotein (HDL) and low density lipoprotein (LDL) to aid in the assessment of liver health at time points specified in Table 4, Table 5, and Table 6. A 3- to 4-hour fasting period is preferred, but not required, prior to blood collection for serum HDL and LDL. All efforts should be made to obtain samples for HDL and LDL consistently for a given subject. For visits in which subjects are receiving study drug, laboratory samples will be collected prior to dosing. Samples will be processed by a central lab to ensure consistency across study sites.

10.4.2. Cardiac Assessments

Details regarding ECG are provided in Section 10.1.3.

10.4.2.1. Creatinine Kinase – Muscle Brain, B-Type Natriuretic Peptide, and Troponin I

Blood will be collected to measure serum creatinine kinase – muscle brain (CK-M/B), B-type natriuretic peptide (BNP), and Troponin I to aid in the assessment of cardiac health at time points specified in Table 4, Table 5, and Table 6. For visits in which subjects are receiving study drug, laboratory samples will be collected prior to dosing. Samples will be processed by a central lab to ensure consistency across study sites.

The study site should indicate to laboratories if the subject is taking high dose biotin supplements (found in multivitamins, biotin supplements, and some supplements to promote hair, skin, and nail growth) to potential risk of assay interference (eg, troponin) (FDA, 2019a).

10.4.3. Skeletal Muscle, Strength, and Physical Functioning Assessments

10.4.3.1. Creatinine Kinase

Blood with be collected to measure serum creatinine kinase (CK) to aid in the assessment of muscle health at time points specified in Table 4, Table 5, and Table 6. For visits in which subjects are receiving study drug, laboratory samples will be collected prior to dosing. Samples will be processed by a central lab to ensure consistency across study sites.

10.4.3.2. Handheld Dynamometry

HHD will be used to assess muscle strength of the following muscle groups: key pinch, grip, elbow flexors, elbow extensors, hip adductors, hip abductors, knee flexors, and knee extensors. As functional ability/disability can vary from patient to patient, the subject only has to complete assessments for the muscle groups for which the clinician administering dynamometry considers safe.

Ultragenyx will conduct formal training with the clinicians administering dynamometry (licensed physical therapist, unless approved by Ultragenyx) to standardize technique and minimize variability. Training must be completed successfully prior to testing any subject for the study. The maximum voluntary isometric contraction against a dynamometer will be used to measure muscle strength. Specialized dynamometers for the measurement of limb, grip, and key pinch strength will be used.

All measurements will be taken bilaterally. The total force (in kg) will be recorded at the time of test administration. The percent predicted values will be calculated after the testing using published normative data (Peters et al., 2011; Bohannon, 1997; NIMS, 1996).

HHD will be performed at specified time points in Table 4, Table 5, and Table 6. HHD should be conducted within a week prior to dosing visits. Additional details regarding HHD are available in the Clinical Evaluator Manual.

10.4.4. Clinician- and Patient-reported Outcomes

10.4.4.1. Patient-Reported Outcomes Measurement Information System

The Patient-Reported Outcomes Measurement Information System (PROMIS) was developed by the National Institutes of Health and uses domain-specific measures to assess patient well-being (NIH, 2015; Broderick et al., 2013). The domain-specific approach is based on the idea that health attributes, such as pain and physical function are not unique to a specific disease. PROMIS Fatigue, Pain Interference, Physical Function, and Global Heath scales will be administered.

The PROMIS questionnaires will be completed at time points specified in Table 4, Table 5, and Table 6. The PROMIS questionnaires should be completed within a week prior to dosing visits. Additional details regarding the PROMIS questionnaires are available in the Clinical Evaluator Manual.

10.4.4.2. 36-item Short-Form Health Survey

The 36-item Short-Form Health Survey version 2 (SF-36v2) health survey captures practical, reliable, and valid information about functional health and well-being from the subject's perspective. The SF-36v2 is a 36-item, self-reported questionnaire designed to assess health-related quality of life in healthy and ill individuals. Specifically, the SF-36v2 assesses physical and mental health based on 8 scaled scores that are the weighted sums of the questions in their section: vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health. Each scale is directly transformed into a 0 to 100 scale on the assumption that each question carries

equal weight. Lower scores indicate more disability, and higher scores indicate less disability, ie, a score of zero is equivalent to maximum disability and a score of 100 is equivalent to no disability.

The SF-36v2 will be completed at time points specified in Table 4, Table 5, and Table 6. The SF-36v2 should be completed within a week prior to dosing visits. Additional details regarding the SF-36v2 are available in the Clinical Evaluator Manual.

10.4.4.3. GNE Myopathy Functional Activities Scale Expanded Version

The GNE Myopathy Functional Activities Scale (GNEM-FAS; also referred to as HIBM-FAS in some studies) was designed to assess the functional impact of changes in muscle strength in GNE myopathy and similar diseases (Mayhew et al., 2017; Skrinar et al., 2013). The scale consists of 3 domains: mobility, upper extremity, and self-care; scores for each domain and a total score will be obtained. Items in the scale assess the subject's ability to independently perform various activities of living that involve self-care, mobility and use of the upper and lower extremities. The original GNEM-FAS was developed for ambulatory patients with GNE myopathy but has since been modified to include additional items to accommodate weaker patients. This modified version is referred to as the GNEM-FAS expanded version and will be used in this study. The scale will be administered by a clinician trained by Ultragenyx (licensed physical therapist, unless approved by Ultragenyx). Training must be completed successfully prior to assessing any subject for the study. The clinicians' role is a combination of observing subjects conducting activities and asking subjects questions to assess performance of these activities.

The GNEM-FAS expanded version will be assessed at time points specified in Table 4, Table 5, and Table 6. The GNEM-FAS expanded version should be completed within a week prior to dosing visits. Additional details regarding the GNEM-FAS expanded version are available in the Clinical Evaluator Manual.

10.4.4.4. Nutrition Diary

Subjects will be asked to maintain a record of daily diet using a diary at time points specified in Table 4, Table 5, and Table 6. For all cohorts, the nutrition diary recordings should be recorded at least 3 consecutive days per week through screening or rescreening, as applicable, and for the 3 consecutive days in the week leading up to each subsequent visit. Qualified Investigator site staff should review nutrition diary data at each visit. Additional details regarding the nutrition diary are available in the Study Reference Manual.

10.5. Pharmacodynamic and Biomarker Assessments

10.5.1. Controlled Fasting Challenge

In RD cohorts, the subject's ability to maintain symptom-free euglycemia during an optional CFC at select sites for select subjects will be assessed at the STV and Week 16 (OL-RD Cohorts) or Week 10 (DB-RD Cohorts) Visits; only subjects who completed the optional CFC at STV complete the CFC at Week 16 or 10. The CFC can be conducted at the hospital during the day or an overnight stay, but the time of day should be consistent within subjects between the STV and Week 16 or Week 10 assessment. For a daytime CFC, an overnight stay in the hospital the night

before might be needed. With either a daytime or overnight CFC, it is critical that appropriate personnel are present to monitor the subject during the CFC and to provide rescue treatment if needed. Special attention should be provided during nursing shift changes.

Before the CFC is conducted, morning cortisol is collected between 6 and 9 am and analyzed by the central lab. This collection can occur on the morning of the CFC or, for the STV CFC, anytime between the ISV and STV. For subjects with suspected hypothalamic-pituitary axis (HPA) dysfunction (eg, those with a history of prolonged steroid treatment), morning cortisol should be reviewed by the Investigator and discussed with the Medical Monitor before deciding if it is safe to conduct the CFC. In this scenario, the morning cortisol sample should be collected at least a week prior to the CFC, providing sufficient time to review the lab results.

Before the CFC begins, a venous catheter is placed for serial blood sampling and to allow for provision of a rescue glucose infusion if needed. A venous blood sample for measurement of glucose, cortisol, glucagon, insulin, C-peptide, and β-hydroxybutyrate will be collected at the beginning (ie, immediately after the provided meal or cornstarch, as applicable, described below), at end of the CFC, and 30 minutes after the end of the CFC. These samples will be sent to a central lab, except glucose which will be analyzed by the local lab.

During the CFC, venous blood samples for STAT analysis of glucose will be collected through an indwelling catheter every 60 minutes (± 5 minutes) until the venous glucose level decreases to ≤ 70 mg/dL (≤ 3.9 mmol/L). Thereafter, venous blood glucose is measured approximately every 30 minutes (± 5 minutes) until the end of the CFC. Venous blood glucose taken during the CFC will be analyzed locally and available within 30 minutes to ensure subject safety. Capillary glucose and ketone measurements using an HHG and ketone meter will also be performed during the CFC at the same time as venous blood glucose or more frequently as needed.

The CFC will end when the subject's glucose level decreases to < 54 mg/dL (< 3.0 mmol/L), when the subject experiences signs and symptoms of hypoglycemia, or the fast reaches 12 (daytime) or 15 (overnight) hours without hypoglycemia, whichever occurs first. During the CFC, subjects may drink water, but cannot ingest any other food or drink. The start and end times of the CFC will be recorded in EDC.

Data from the HHG will be uploaded via the study provided laptop. Data from venous blood glucose and capillary ketones taken during the CFC will be entered into the EDC. Subjects should continue to wear their CGM device throughout the fasting challenge. Readings from the CGM device during the CFC may alert the site to a potentially low glucose value that would require confirmation by capillary and venous blood glucose.

At the end of the CFC, the subject's hypoglycemia should be treated per the institution's standard procedure, which may include providing the subject with a meal, cornstarch, or IV glucose, if medically indicated.

Daytime CFC

If the CFC is conducted during the day, the study site will provide breakfast for the subject. The breakfast meal will be personalized for each subject and will include a target carbohydrate range that should be as close as possible to the subject's usual breakfast meal based on their current nutrition prescription; for the Week 16 (OL-RD Cohorts) or 10 (DB-RD Cohorts) CFC, the breakfast meal will be identical to the breakfast consumed for the STV CFC. Subjects will be

instructed to consume the entire breakfast meal and minimize activity after they finish eating breakfast until the end of the CFC. Breakfast meal stop time and its full nutrient composition will be recorded by the subject in the nutrition diary with the assistance of the study dietitian as needed. If a subject is taking a morning dose of cornstarch or protein supplement with breakfast, they will be given their usual morning dose at the usual time but no more than 4 hours after breakfast. The amount of cornstarch and protein to administer at the Week 16 or 10 CFC should be the same as what the subject received at their STV CFC. The time and amount will also be recorded by the subject in the nutrition diary with the assistance of the study dietitian as needed. The CFC starts when the subject finishes breakfast and the associated cornstarch and/or protein dose, as applicable.

Overnight CFC

If the CFC is conducted overnight, the study site will provide dinner for the subject. The dinner meal will be personalized for each subject and will include a target carbohydrate range that should be as close as possible to the subject's usual dinner meal based on current nutrition prescription. for the Week 16 (OL-RD Cohorts) or 10 (DB-RD Cohorts) CFC, the dinner meal will be identical to that of the STV CFC. Subjects will be instructed to consume the entire dinner meal and minimize activity after they finish eating dinner until the end of the CFC. Dinner meal stop time and its full nutrient composition will be recorded by the subject in the nutrition diary with the assistance of the study dietician as needed. If a subject is taking cornstarch or protein supplement, they will be given their usual oral dose at the usual time but no more than 4 hours after dinner. The amount of cornstarch and/or protein to administer at the Week 16 or 10 CFC should be the same as what the subject received at their STV CFC. The time and amount will also be recorded by the subject in the nutrition diary with the assistance of the study dietician as needed. If a subject does not a receive cornstarch or protein supplement before going to bed, the CFC starts when the subject finishes dinner. The CFC starts when the subject finishes dinner and the associated cornstarch and/or protein dose, as applicable.

10.5.2. Ketones

In addition to urine ketones assessed in urinalysis (Section 10.1.5 and Appendix 3), blood (serum for SAD cohorts and capillary for RD cohorts) will be analyzedto evaluate the PD effect of UX053 on ketosis at specified time points in Table 4, Table 5, and Table 6. For visits in which subjects are receiving study drug, blood or capillary measurements will be collected prior to dosing.

10.5.3. Continuous Glucose Monitoring

At the Screening Visit for the SAD cohort and ISV for the DB-RD cohort, each subject will receive a CGM and HHG device and be trained on how to use the device. For SAD cohorts, the CGM device will be worn from the Screening Visit until the End of Study (EOS) I D90/ET Visit (Table 4). For OL-RD cohorts, the continual glucose monitor will be worn from the OL-RD rescreening ISV to the Week 24 Visit, and for the week prior to the Week 36 and EOS II W48/ET Visits (Table 5). For DB-RD cohorts, the CGM will be worn for from ISV to the Week 24 Visit, and for the week prior to the Week 36 and EOS III W48/ET Visits (Table 6).

If the CGM device alarm notifies a subject that their blood sugar is abnormally high or low or the subject suspects their blood sugar is abnormally high or low, the subject will need to check their

blood sugar with a HHG that will be provided by Ultragenyx during screening (or rescreening, as applicable). If action is taken to rectify abnormal blood glucose levels, the event should be recorded as an AE (Appendix 1 and Appendix 2).

When CGM data is being collected, all subjects will connect their CGM and HHG devices to a study-provided laptop weekly and upload CGM and HHG data. At remote visits, in conjunction with assessments performed by a home health nurse, the site will contact subjects by telephone or videoconference to confirm the CGM device is properly in use and data was uploaded; answer any questions regarding or help troubleshoot (if necessary) CGM device use; and review CGM data, HHG data (when applicable), and nutrition diary entries; outside of scheduled visits, these telephone calls will occur as needed. At in-clinic visits, a conversation between the site and the subject should occur to ensure CGM compliance and troubleshoot if needed.

Additional details regarding the CGM device are available in the Study Reference Manual.

10.5.4. Hemoglobin A1C

Hemoglobin A1C integrates average blood glucose over a 3-month period and can be used to monitor more chronic changes in blood glucose. Blood will be collected to measure hemoglobin A1C at specified time points in Table 4, Table 5, and Table 6. Samples will be processed by a central lab to ensure consistency across study sites.

10.5.5. Glucose Tetrasaccharide

Glc_4 is a limit dextrin of the glucose polymer amylopectin and is elevated in GSD III (Halaby et al., 2019).

Urine will be collected for assessment of Glc_4 to evaluate a biomarker of disease activity in GSD III at specified time points in Table 4, Table 5, and Table 6. All efforts should be made for urine collection to be consistently taken from the first morning void.

10.5.6. *AGL* Gene Sequencing

Blood will be collected for *AGL* mutation analysis for all qualified subjects, even if the subject or a family member has a confirmed *AGL* mutation, at time points in Table 4 and Table 6. For each subject, both alleles of the *AGL* gene will be analyzed by a central laboratory for known pathogenic or likely pathogenic mutations. Results will be used for research purposes to better understand the genotype-phenotype relationships in affected individuals with GSD III.

10.5.7. Exploratory Future Use of Blood

Blood will be collected and processed into serum, plasma, white blood cells, and red blood cells for optional, non-protocol-required additional future research at time points in Table 4, Table 5, and Table 6. These additional samples will be used to assess novel emerging biomarkers of GSD III and/or UX053 and the relationship to GSD III presentation or prognosis. For visits in which subjects are receiving study drug, blood will be collected prior to dosing.

10.6. Appropriateness of Measurements

This is a FIH study designed to assess the safety, PK, PD, and preliminary efficacy of UX053 in patients with GSD III. The safety parameters to be evaluated in this study include standard assessments such as recording of medical history, AEs, SAEs, physical examination, vital signs, serum chemistry, concomitant medications, and other clinical and laboratory procedures.

The efficacy endpoints to be evaluated in this study examine liver health, cardiac health, muscle health, strength, and patient- and clinician-reported outcomes. Exploratory assessments include potential biomarkers of disease activity that can be used to monitor PD effects of UX053. These efficacy and exploratory assessments are standard measures used to identify, assess, and monitor the signs and symptoms of GSD III. Where possible, timing of assessments has been coordinated with standard safety laboratory tests to minimize risk of discomfort and avoid unnecessary duplication of testing. Nutrition diaries will also be used to characterize disease management and monitor care throughout the study.

11. STATISTICAL CONSIDERATIONS

A full description of the analysis details will be provided in the SAP.

11.1. Sample Size Determination

The sample size for this study is based on practical considerations and is consistent with a FIH study in a rare disease population. The study plans to enroll approximately 18 subjects (assuming Cohorts 1-3S and DB-1-3R are initiated), with a minimum of 6 subjects in the SAD cohorts and 12 subjects in the DB-RD cohorts, with the option to enroll additional subjects in any cohort to further characterize safety or PD findings. With at least 6 subjects receiving UX053 in the SAD cohorts, there is an 74% chance of detecting a more common AE given the TEAE has a true incidence of 20%. If at least 18 subjects receive UX053 in both SAD and RD cohorts, there is an 85% chance of detecting a TEAE with true incidence of 10%.

Subjects will be replaced at Ultragenyx's discretion only if they have not received study drug. Subjects will not be replaced if they have received any study drug.

11.2. Analysis Sets

The Safety Analysis Set will include all enrolled subjects, defined as subjects deemed eligible for treatment at D0 in the SAD cohorts, and those randomized in the DB-RD cohorts, and those who continue into OL-RD from SAD cohorts. A Per Protocol (PP) Analysis Set, consisting of subjects who complete the study without major protocol deviations that could compromise PD or efficacy assessments may be analyzed as described in the SAP. The PK Analysis Set is a subset of subjects in the Safety Analysis Set who have at least 1 evaluable IP concentration. The PK Analysis Set will be used for the analysis of PK endpoints.

11.3. Planned Methods of Analysis

This section is a summary of the planned statistical analyses of the most important endpoints.

11.3.1. General Principles

A general description of the statistical methods to be used to analyze the study endpoints is outlined below. The analyses planned in this protocol will be expanded in the SAP to include detailed description of the analyses.

Descriptive statistics will be provided for selected demographics, safety, PK, and PD data. After review, data for subjects receiving placebo may be combined across all cohorts to form a pooled placebo group. If a sufficient number of subjects with different disease subtypes are enrolled, subgroup summaries by disease subtype will be provided. Continuous variables will be summarized by number of subjects and mean, SD and/or SE, median, minimum, and maximum values. Categorical variables will be summarized by number and percentage of subjects. Data will be summarized with descriptive statistics for the Screening Period for the DB-RD cohorts.

COVID-19 contingency measures and trial subjects affected by COVID-19 will be listed.

Although they are eligible, it is expected that few patients with GSD IIIb, IIIc, or IIId will enroll. Subjects with GSD IIIb/c/d will participate in all assessments, including muscle imaging and

function tests, and will be included in all safety analyses. Sensitivity analyses for cardiac and skeletal muscle endpoints may exclude patients with GSD IIIb/c/d as described in the SAP.

11.4. Safety Analyses

Safety variables including all AEs, SAEs, TEAEs, serious TEAEs, related TEAEs, safety laboratory assessments (full list available in Appendix 3), vital signs, weight, physical examination, clinical laboratory evaluations, and ECGs will be summarized for all subjects throughout the study and by treatment and cohort. Incidence and severity of IRRs and hypersensitivity reactions will also be summarized for all subjects throughout the study and by treatment and cohort. All AEs occurring from the onset of the study treatment infusion, and within 4 hours following the end of the infusion, regardless of the Investigator's assessment of whether or not the event was related to study drug administration will be considered an IRR.

All AEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.0. The frequency and severity of all TEAEs, SAEs, and clinically relevant laboratory and ECG abnormalities will be reported by System Organ Class (SOC), by preferred term, and by relationship to study drug. All AEs reported with onset after the first dose of study drug (ie, TEAEs) will be included in the analysis. All SAEs will also be reported by relationship to study drug as reported by Investigator, seriousness, and outcome. For all AEs, SAEs, and treatment-related AEs, SAEs, the percentage of subjects who experienced at least 1 occurrence of the given event will be summarized for all subjects in the SAD cohorts and by treatment and cohort for the RD cohorts. Data summaries, listings, and narratives will be provided for subjects who died, discontinued treatment or withdrew from study due to an AE, or experienced SAEs, and any study procedure-related AEs.

Clinical laboratory data will be summarized by the type of laboratory test and by changes from baseline. Reference ranges and abnormal results (specified in the SAP) will be used in the summary of laboratory and ECG data. The frequency and percentage of subjects who experience abnormal clinical laboratory results (ie, outside of reference ranges) and/or clinically significant abnormalities (as determined by the Investigator) will be presented for each clinical laboratory measurement, including for Hy's Law analysis (ALT, AST, total and direct bilirubin, ALP). Shift tables may be provided for changes from baseline in selected chemistry and hematology laboratory parameters. Listings of abnormal vital signs and physical findings will be provided. The SAP will provide additional details on the planned safety analyses. Several safety laboratory tests will also be examined for efficacy (Section 11.7).

11.5. Pharmacokinetic Analyses

PK parameters will be derived by non-compartmental analysis. PK parameters to be estimated may include, but not limited to, the following: T_{max}, C_{max}, AUC_{last}, AUC_{inf}, AUC_{tau}, R_{AUC}, T_{last}, $T_{1/2}$, CL, and V_{ss}. PK parameters will be descriptively summarized by dose levels and time in SAD and RD, respectively. The SAP will provide additional details on PK analyses.

11.6. Anti-drug Antibody Analyses

The incidence and titer for ADA to CCI and GDE will be evaluated and summarized for each subject at time points specified in Table 4, Table 5, and Table 6. Samples may be stored for further assessment to evaluate the presence of neutralizing antibodies.

11.7. Efficacy Analyses

Efficacy parameters will be summarized at screening (or rescreening, as applicable) and baseline (or both), and at each observed time point that the assessment is collected thereafter, as specified in Table 4, Table 5, and Table 6; change from screening (or rescreening, as applicable) or baseline (or both) will also be summarized where applicable. Several laboratory tests examined for safety will also be considered for efficacy (Section 11.4). Additional details regarding tertiary and exploratory endpoint analysis are available in the SAP.

11.8. Nutrition Diary

Data from the nutrition diary will be summarized with descriptive statistics. Additional details regarding analysis of the nutrition diaries are available in the SAP.

11.9. Exploratory Analyses

Details regarding exploratory analyses are available in the SAP.

11.10. Planned Subgroup Analyses

If a sufficient number of subjects with different disease subtypes are enrolled, subgroup summaries by disease subtype will be provided. Additional details regarding potential subgroup analysis are available in the SAP.

11.11. Planned Analyses

Analyses are planned for each data review described in the study design (Section 7.1). For the SAD cohorts, Ultragenyx will review at least 2 weeks of safety data for all subjects within a cohort before deciding to proceed with the next SAD cohort. For the RD cohorts, an unblinded DMC will review either at least 6 weeks of safety from at least 4 subjects in a DB-RD cohort or 12 weeks of safety data from at least 3 subjects in an OL-RD cohort (whichever is available first) before providing a recommendation for proceeding with repeat dosing at the next dose level. All available data from prior cohorts will also be reviewed when considering proceeding with new cohorts.

11.12. Data Monitoring Committee

An independent DMC will be constituted with experts in metabolic liver disease, GSD III, and/or immune activation and will act in an advisory capacity to Ultragenyx. Additionally, at least 1 of the DMC members will have expertise concerning statistical methods used in the design and analysis of clinical trials.

The DMC will meet and review study data at the frequency defined and in accordance with the scope and objectives set forth in the DMC Charter. The DMC may provide advice to Ultragenyx regarding the safety of subjects, the ethics of the study and the continuing scientific validity of the study. The DMC may also make recommendations to Ultragenyx concerning continuation, termination or other modifications of the study based on their review of data. Further details regarding the DMC can be found in the DMC charter, which will be available prior to the administration of IP.

Ultragenyx will review the DMC recommendations and determine necessary actions that will be communicated accordingly to all stakeholders, eg, Regulatory Authorities, IRBs/IECs, and Investigators.

12. REFERENCES

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13. APPENDICES

APPENDIX 1. SAFETY DEFINITIONS AND ASSESSMENTS

Adverse Event (AE)

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) products.

Pre-existing diseases or conditions will not be considered AEs unless there is an increase in the frequency or severity, or a change in the quality, of the disease or condition. Worsening of a pre-existing condition is considered an AE.

Any abnormal laboratory test results (hematology, clinical chemistry, or urine) or other safety assessments (eg, vital sign measurements), including those that worsen from screening/baseline that are felt to be clinically significant in the medical and scientific judgment of the Investigator are to be recorded as AEs.

Treatment-emergent AE (TEAE)

A TEAE is defined as any AE not present prior to the initiation of the drug treatment or any AE already present that worsens in either intensity or frequency following exposure to the drug treatment.

Serious Adverse Event (SAE)

An SAE is an AE that meets any of the following criteria in the view of either the Investigator or Ultragenyx:

- Death
- Life-threatening
  - *A life-threatening AE is an event that places the patient or subject at immediate risk of death. It does not include events that if it had occurred in a more serious or severe form might have caused death. For example, drug induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug induced hepatitis can be fatal.*
- Inpatient hospitalization or prolongation of existing hospitalization
  - *Hospitalization is defined by Ultragenyx as a full admission to hospital for a period of 24 hours or longer for diagnosis and treatment. This includes prolongation of an existing inpatient hospitalization. Examples of visits to a hospital facility that do not meet the serious criteria for hospitalization include:*
    - *Emergency room visits (that do not result in a full hospital admission)*
    - *Preplanned or elective procedures prior to study enrollment (eg, outpatient surgery)*
    - *Protocol procedures*

  - *Hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.*
  - *AEs requiring hospitalization should be considered SAEs. Hospitalization planned prior to study enrollment (eg, for elective surgery or routine clinical procedures) that are not the result of AE (eg, elective surgery for a pre-existing condition that has not worsened) need not be considered AEs or SAEs. If anything untoward is reported during the procedure, that occurrence must be reported as an AE, either 'serious' or 'non-serious' according to seriousness criteria described above.*

- Disability/Incapacity
  - *An AE is disabling or incapacitating if it results in substantial and/or permanent disruption of the subject's ability to carry out normal life functions.*
- Congenital anomaly/birth defect not present at screening
- Important Medical Events that may not result in death, be immediately life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the criteria listed in the definition. Examples of such events are:
  - Intensive treatment in an emergency room or at home for allergic bronchospasm
  - Blood dyscrasias that do not result in inpatient hospitalization
  - Development of drug dependency or drug abuse
  - Emergency room visits requiring medical intervention related to the study or study procedures, where subjects, due to hospital capacity, could not be admitted (eg, due to coronavirus 2019 [COVID-19])

Participation-emergent Adverse Events

These events may be related to study participation or biological specimen collection and will be reported by the Investigator and noted as such in the case report form (CRF) as related to study intervention.

Overdose

An overdose is defined as a known deliberate or accidental administration of investigational drug, to or by a study subject, at a dose above that which is assigned to that individual subject according to the study protocol. All cases of overdose (with or without associated AEs) will be documented in the CRF.

Assessment of Severity

The severity or intensity of an AE refers to the extent to which an AE affects the patient's daily activities. The severity of all AEs will be graded using the Common Terminology Criteria for

Adverse Events (CTCAE) version 5.0, published on November 27, 2017, unless the AE is Cytokine Release Syndrome (CRS), in which case the AE will be graded by the American Society for Transplantation and Cellular Therapy (ASTCT) Consensus Grading for CRS (Lee et al., 2019).

Table 13: American Society for Transplantation and Cellular Therapy Consensus Grading for Cytokine Release Syndrome

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever[a]	Temperature ≥ 38°C	Temperature ≥ 38°C	Temperature ≥ 38°C	Temperature ≥ 38°C
		with		
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
		And/or[b]		
Hypoxia	None	Requiring low-flow nasal cannula[c] or blow-by	Requiring high-flow nasal cannula[c], facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (eg, CPAP, BiPAP, intubation and mechanical ventilation)

[a] Fever is defined as temperature ≥ 38°C not attributable to any other cause. In patients who have CRS then receive antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

[b] CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.

[c] Low-flow nasal cannula is defined as oxygen delivered at ≤ 6 L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at > 6 L/minute.

Note: Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.

BiPAP, bilevel positive airway pressure; CPAP, Continuous positive airway pressure; CRS, cytokine release syndrome; CTCAE, Common Terminology Criteria for Adverse Events.

Source: (Lee et al., 2019).

If an AE cannot be graded using the CTCAE criteria, it should be graded using the following definitions:

- Mild (Grade 1): Subject has awareness of signs or symptoms, but easily tolerated and are of a minor irritant type, causing no loss of time from normal activities. Symptoms do not require therapy or a medical evaluation; signs and symptoms are transient.
- Moderate (Grade 2): Events introduce a low level of inconvenience or concern to the subject and may interfere with daily activities, but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.
- Severe (Grade 3): Events interrupt the subject's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating.

- Life-threatening (Grade 4): Events that place the subject at immediate risk of death or are disabling.
- Death (Grade 5): Events that result in death.

Note: The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious" which is based on subject/event outcome or action criteria and is usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Assessment of Causality

The Investigator will make a causality assessment about the relationship of each AE to study drug. Treatment-related conditions must be distinguished from disease-related conditions.

The Investigator should determine the causality (relation to the study drug) based on his/her clinical experience and on the information given in the Investigator's Brochure. The causal relationship of all AEs to the study drug will be judged as either related (which includes possibly, probably or definitely related) or not related (which includes unlikely or doubtfully related).

To ensure consistency of AE and SAE causality assessments, Investigators should apply the following general guideline:

- A **related** causal relationship means that there is at least a reasonable possibility that the event is caused by the study drug or the research procedures. There may be a reasonable possibility of a causal relationship between study drug/Investigational Product (IP) and the AE when:
    - The event follows a reasonable temporal sequence post IP administration
    - The event is known to be or could be a response to the IP, based upon pre-clinical or clinical data with the product or similar products
    - The event could not be explained by the subject's primary disease including progression/expression of the disease state, other concurrent or underlying illness, and/or prior/concomitant therapies
    - Positive dechallenge: the event resolves or improves after discontinuation of IP (when this information is available)
    - Positive rechallenge: the event reappears or worsens when dosing with IP is resumed after an interruption (when this information is available)
- **Not related** means there is unlikely to be a reasonable possibility of a causal relationship between the event and study drug/IP or the research procedures, and/or that there is a clear causal relationship between other conditions and the AE. There is not likely to be a reasonable possibility of a causal relationship between study drug/IP and the AE when:

- The event does not follow a reasonable temporal sequence post IP administration
- There are no data with the IP or similar products, suggesting the event occurs or may occur with the IP
- There is a more likely alternative etiology, such as subject's underlying primary disease including disease progression or expression, other concurrent or underlying illness, or prior/concomitant therapies
- Negative de-challenge: the event does not improve after discontinuation of IP (when this information is available)
- Negative re-challenge: the event does not worsen after interruption of IP when dosing is resumed after an interruption (when this information is available)

Note: The Investigator's assessment of causality for individual AEs is part of the study documentation process and will be recorded in the patient's medical record, CRF, and SAE form, if applicable. AEs recorded without the Investigator's assessment of the relationship to study drug will be followed up until causality is assigned.

Suspected Unexpected Serious Adverse Reaction (SUSAR) means a SAE that occurs in a clinical trial subject, which is assessed by the Investigator or Ultragenyx as being serious and unexpected based on Reference Study Information (eg, Investigators Brochure [IB]), and as having a reasonable possibility of a causal relationship with the study drug/IP.

Urgent Safety Measure means any measure taken to protect the subjects of a clinical trial against an immediate hazard to their health or safety.

APPENDIX 2. ADVERSE EVENT COLLECTION: ELICITING, RECORDING, AND REPORTING

Table 14: Collection Period & Reporting Timeframe: Adverse Event & Other Safety Information Reporting

Event Type	Screening Period (including nutrition optimization and standardization), SAD cohorts, RD cohorts	Reporting Timeframe	Report to
SAE	**Start:** From the time the subject signs the ICF **End:** 28 days following the final dose of study drug or the EOS I (SAD cohorts) and EOS II or EOS III (RD cohorts) final study visit, whichever occurs later Suspected related SAEs can be reported at any time following the EOS I/II visit	Report all SAEs within 24 hours of site Investigator, designee or site personnel's knowledge of the event.	**Electronic Data Capture (primary)** **Ultragenyx** **(back-up if Electronic Data Capture is down or not available)** email: ultragenyx@primevigilance.com **Fax:1 (415) 930-4033**
AE	**Start:** From the time the subject signs the ICF **End:** 28 days following the final dose of study drug or the EOS I (SAD cohorts) and EOS II or EOS III (RD cohorts) final study visit, whichever occurs later	Report all non-serious AEs within a target of 3 business days of site Investigator, designee or site personnel's knowledge of the event.	**Electronic Data Capture**
Pregnancy (includes Partner pregnancy)	**Start:** From the time the subject signs the ICF **End:** 28 days following the final dose of study drug or the EOS I (SAD cohorts) and EOS II or EOS III (RD cohorts) final study visit, whichever occurs later	Report all pregnancies within 24 hours of site Investigator, designee or site personnel's knowledge of the event. Request consent to follow partner pregnancy (if applicable). Follow pregnancy until outcome is known (live birth, stillbirth, spontaneous abortion, elective termination, etc.).	Ultragenyx email: ultragenyx@primevigilance.com **Fax:1 (415) 930-4033**

AE, adverse event; EOS, end of study; ICF, informed consent form; RD, Repeat Dose; SAD, Single Ascending Dose; SAE, serious adverse event.

Table 15: Safety Contact Information

Global Drug Safety & Pharmacovigilance SAE and Pregnancy Reporting	Medical Monitor
Fax: 1 (415) 930-4033 email: ultragenyx@primevigilance.com	PPD 60 Leveroni Ct. Novato, CA 94949 PPD

Eliciting Adverse Events

A consistent methodology of non-directive questioning for eliciting adverse events (AEs) at all patient evaluation time points should be adopted. Examples of non-directive questions include:

"Since the last visit, has the patient experienced any health problems?"

"Have there been any new or changed health problems since you were last here?"

Recording AEs and SAEs

All AEs (ie, any new condition or worsening in severity or frequency of a preexisting condition) experienced by the patient within the protocol defined timeframes must be promptly documented on the case report form (CRF) and serious adverse event (SAE) form if applicable. This includes any AEs or SAEs considered associated with study protocol required procedures. For clinically significant worsening of a preexisting condition from Baseline the changes will be documented as AEs on the CRF. Clinical significance is defined as any variation in signs, symptoms, or testing that has medical relevance and may result in an alteration in medical care. The Investigator will continue to monitor the patient until the assessment returns to Baseline or until the Investigator determines that follow-up is no longer medically necessary.

The Investigator is responsible for evaluating all AEs, obtaining supporting documents, and ensuring documentation of the events is adequate. Details of the AEs must include severity, seriousness, relationship to study drug, duration (dates of onset and resolution), and outcome.

In addition, the Investigator should report any AE or SAE they are made aware of that occurs after the end of the study and that is believed to have a reasonable possibility of being associated with study drug.

Diagnosis versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF and SAE form (if applicable) rather than individual signs and symptoms. Whenever medically appropriate, the Investigator should group signs or symptoms that constitute a single diagnosis into a unifying event term. For example, record "liver failure" rather than jaundice, asterixis, and elevated transaminases, or record "upper respiratory tract infection" rather than cough, rhinitis, and sneezing. However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE on the CRF and SAE form (if applicable). If a diagnosis is subsequently established, it should be reported as follow-up information by revising the CRF and SAE form (if applicable). Vague, nonspecific AE terms

such as “erythema,” “rash,” or “lump on head” should be avoided and more specific information should be provided, such as “erythematous macule on right leg,” “allergic dermatitis,” or “scalp cyst.”

Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as the AE on the CRF and, if also serious, on the SAE form. However, medically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution between patient evaluation time points. Such events should only be recorded once in the CRF unless their severity increases. If a persistent AE becomes more severe, it should be recorded again on the CRF.

A recurrent AE is one that occurs and resolves between patient evaluation time points and subsequently recurs. All recurrent AEs should be recorded individually on the CRF.

Abnormal Laboratory Values

Only clinically significant laboratory abnormalities in the opinion of the Investigator are to be recorded as AEs on the CRF and SAE form (if applicable). For example, abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, or further diagnostic investigation may be considered clinically significant by the Investigator.

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (eg, alkaline phosphatase and bilirubin 5 × upper limit of normal (ULN) associated with cholecystitis), only the diagnosis (eg, cholecystitis) needs to be recorded on the CRF and the SAE form (if applicable).

If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as “hyperkalemia.”

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs on the CRF and SAE form (if applicable), unless their severity, seriousness, or etiology changes.

Reporting Serious Adverse Events

Any AE that meets SAE criteria must be reported to Ultragenyx or its designee within 24 hours of the site Investigator, designee or site personnel’s knowledge of the event. Regardless of causality, all SAEs must be reported during the time period. All SAEs must also be recorded in the patient’s source documentation and on the CRF. Copies of discharge summaries, consultant reports, and any other relevant documents should be provided with the SAE report when available.

If follow-up is obtained or requested by Ultragenyx and/or Medical Monitor, the additional information should be sent within 24 hours of knowledge.

Refer to Safety Contact Information for SAE reporting (Appendix 2).

Reporting of Pregnancy in Subject or Partner

For protocols requiring pregnancy reporting, any reported pregnancy of a subject or a subject's partner that occurs during participation in the study will be monitored for the full duration of the study and/or followed until the outcome of the pregnancy is known. In the event of a pregnancy in the partner of a subject, the Investigator should make every effort to obtain the female partner's consent for release of protected health information.

Pregnancy in a subject or subject's partner, complications of the pregnancy and the outcomes of pregnancy should be reported to the Ultragenyx or designee within 24 hours of site Investigator, designee or site personnel's knowledge of the event.

Pregnancies will be reported by completing and submitting Pregnancy Notification Form to Ultragenyx or designee.

Pregnancy outcomes will be reported by completing and submitting Pregnancy Outcome Form to Ultragenyx or designee.

The following pregnancy outcomes should always be classified as serious and reported to Ultragenyx or designee within 24 hours by completing and submitting the SAE and Pregnancy Outcome Form:

- Spontaneous abortion/Miscarriage
- Therapeutic abortion
- Ectopic pregnancy
- Fetal death/Still birth
- Molar pregnancy
- Birth defect/Congenital anomaly

Reporting Suspected Unexpected Serious Adverse Reactions

Ultragenyx or its designee will submit suspected unexpected serious adverse reactions (SUSARs) to appropriate Regulatory Authorities (including Competent Authorities in all Member States concerned), Institutional Review Boards/Independent Ethics Committees (IRBs/IECs), and Investigators as per local laws and regulations. Fatal and life-threatening SUSARs will be submitted no later than seven calendar days of first knowledge of the event and follow-up information submitted within an additional eight days. All other SUSARs will be submitted within 15 calendar days of first knowledge of the event.

The Investigator will notify the IRBs/IECs of SUSAR, in accordance with IRB/IEC requirements and local laws and regulations.

Non-SUSARs will be maintained in the Ultragenyx global safety database and provided in annual safety reports and/or other aggregate periodic summary reports to Regulatory Authorities and IRBs/IECs per local laws and regulations.

Reporting Urgent Safety Measures

Investigators are required to report any urgent safety measures taken to protect the safety or welfare of subject to Ultragenyx or its designee within 24 hours. Ultragenyx or its designee will inform the Regulatory Authorities, IRBs/IECs, and other investigators of any events (eg, change to the safety profile of Investigational Product (IP), major safety findings) that may occur during the clinical study that do not fall within the definition of a SUSAR but may affect the safety of subjects participating in the clinical study, as required, in accordance with applicable laws and regulations.

The Investigator will notify the IRBs/IECs of urgent safety measures, in accordance with IRB/IEC requirements and local laws and regulations. A copy of this notification must be provided to Ultragenyx or its designee.

Non-SUSARs will be maintained in the Ultragenyx global safety database and provided in annual safety reports and/or other aggregate periodic summary reports to Regulatory Authorities and IRBs/IECs per local laws and regulations.

APPENDIX 3. LABORATORY ASSESSMENTS

Chemistry	**Hematology**	**Urinalysis**	**Additional Labs**
Alanine aminotransferase (ALT)	Hematocrit	Appearance	**Blood Collection**:
Alkaline phosphatase (ALP)	Hemoglobin	Color	Alternative complement activity (AH50)
Amylase	Mean corpuscular hemoglobin (MCH)	pH	B-type natriuretic peptide (BNP)
Aspartate aminotransferase (AST)	MCH concentration (MCHC)	Specific gravity	Classical pathway complement activity (CH50)
Bicarbonate	Mean corpuscular volume (MCV)	Bilirubin	C-reactive protein (CRP)
Bilirubin (direct and total)	Platelet count	Creatinine	Creatinine kinase (CK)
Blood urea nitrogen (BUN)	Red blood cell (RBC) count	Glucose	Creatinine kinase-muscle/brain (CK-M/B)
Calcium	Reticulocyte count	Hemoglobin	D-dimer
Chloride	White blood cell (WBC) count	Ketones	Fibrinogen
Cholesterol (total)	WBC differential	Nitrite	Hemoglobin A1C
Creatinine	Basophil count (absolute and %)	Protein	High density lipoprotein (HDL)
Gamma-glutamyl transferase (GGT)	Eosinophil count (absolute and %)	Urobilinogen	Interleukin-6 (IL-6)
Glucose	Lymphocyte count (absolute and %)	Pregnancy (if applicable)	Ketones
Lactate dehydrogenase (LDH)	Monocyte count (absolute and %)		Low density lipoprotein (LDL)
Lipase	Neutrophil count (absolute and %)		Prothrombin time/international normalized ratio (PT/INR)
Magnesium	Complete blood count with differential (CBC/diff)		Troponin I
Phosphorus			Soluble complement 5b-9 (sC5b-9)
Potassium			**Urine Collection**:
Protein (albumin and total)			Glucose tetrasaccharide (Glc_4)
Sodium			
Triglycerides			

Note: table does not include blood collection for PK (ATX95, *AGL* mRNA), anti-drug antibodies, *AGL* variant analysis, and serum and plasma for future use; these assessments are measured as specified in Table 4, Table 5, Table 6, and Table 8.

APPENDIX 4. CONTRACEPTION GUIDANCE

The guidance below applies for development products with potential or unknown reproductive toxicity (for products in clinical trials, where pre-clinical data are indicative of risk or have not yet been completed).

Female subjects of childbearing potential who are heterosexually active must consent to use a highly effective method of contraception as listed below to prevent pregnancy over the time period required for product levels to decrease to a concentration that is no longer considered relevant for human teratogenicity/fetotoxicity. This time period is generally determined by the pharmacokinetic properties of the study product and non-clinical reproductive toxicity data and is defined in the protocol or product Investigator Brochure (IB).

Highly effective contraceptive methods are those with a failure rate of less than 1% per year when used consistently and correctly.

Female subjects of childbearing potential are defined as having reached menarche prior to or during the study. Females not of childbearing potential include those who have not experienced menarche, are postmenopausal (defined as having no menses for at least 12 months without an alternative medical cause) or are permanently sterile due to having total hysterectomy, bilateral salpingectomy, or bilateral oophorectomy.

- A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient and multiple measurements are needed.

Male subjects who are heterosexually active with female partners of childbearing potential must consent to use one of the highly effective methods of contraception listed below to prevent pregnancy over the period defined in the protocol or product Investigator Brochure. The time period required is generally determined by the pharmacokinetic properties of the study product and non-clinical reproductive toxicity data.

Highly effective methods of contraception (CTFG, 2014) include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (eg, oral, intravaginal, transdermal)
- Progestogen-only hormonal contraception associated with inhibition of ovulation (eg, oral, injectable, implantable)
- Intrauterine device or intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Male sterilization, also called vasectomy
- Sexual abstinence is defined as refraining from heterosexual intercourse during the entire study period associated with risk of the Investigational Product (IP), other study treatments or procedures

- The reliability of sexual abstinence should be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.
- Abstinence is only considered a highly effective method of contraception if practiced consistently for the entire period of exposure to IP or other study treatments.
- Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea methods are not acceptable methods of contraception.

Notes: Barrier methods (such as a condom used with or without a spermicide or a diaphragm or cervical cap used with a spermicide) used alone are NOT highly effective contraception methods.

Additional Requirement if IP is an advanced therapy medicinal product (eg, gene therapy), a condom with spermicide is also required to be used by all sexually active males in the study in order to prevent potential transmission of the vector via seminal fluid.

APPENDIX 5. ETHICS AND CONDUCT

This protocol is written in accordance with the principles established by the 18th World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects and subsequent amendments and clarifications adopted by the General Assemblies (World Medical Association, 2013).

Ultragenyx and Investigator will ensure the study described in this protocol is conducted in compliance with those principles and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and applicable local law, eg, Food and Drug Administration (FDA) regulations, European Union (EU) regulations, and any other local ethical and regulatory requirements ("Applicable Study Conduct Obligations"). Should a conflict arise, Ultragenyx and Investigator will follow whichever Applicable Study Conduct Obligations affords the greater protection to the subject(s).

Institutional Review Board or Independent Ethics Committee and Competent Health Authority

The Institutional Review Boards/Independent Ethics Committees (IRB/IEC) must be a properly constituted board or committee operating in accordance with Applicable Study Conduct Obligations and should safeguard the rights, safety, and well-being of all study subjects. Before screening any subject, the IRB/IEC, and as applicable Competent Health Authority, must review and approve this protocol and if applicable, the associated assents and informed consent forms (ICFs), agreed to by the study site (ie, Institution) and by Ultragenyx. Further, IRB/IEC and Competent Health Authority approval of any protocol amendments must be received before any of the changes outlined in the amendments are put into effect, unless there is a need to protect subject safety. In that case and with prior IRB/IEC approval/favorable opinion, the Investigator may deviate from the protocol to eliminate an immediate hazard(s) to study subjects and will immediately notify the chair of the IRB/IEC and Ultragenyx of the amendment.

If Investigational product (IP) is provided under the protocol, IP will not be shipped to the study site until Ultragenyx or its designee has received a copy of the letter or certificate of approval/favorable opinion from the IRB/IEC, all Ultragenyx-required documents, and, where required, the Competent Health Authority authorization/approval of the protocol. If IP is not provided under the protocol, this paragraph and the section on IP accountability do not apply.

Before releasing advertisements or solicitations to the public for subject enrollment in the study, such advertisements or public solicitations must be submitted to Ultragenyx or its designee for review and approval. After receiving Ultragenyx approval for the advertisements or public solicitations, the Investigator must submit them to his/her IRB/IEC for review and approval before using them.

Subject Information and Consent

The contents of the ICF and any assents for minors, as well as the method of obtaining and documenting informed consent must comply with Applicable Study Conduct Obligations.

Ultragenyx or its designee will provide the Investigator written informed consent(s), which includes assents for minors. Any revisions to the ICF must be reviewed and approved by Ultragenyx before submission to the IRB/IEC for its approval. The study site agrees not to begin enrolling subjects until the IRB/IEC approval is complete.

Adequate consent for access to, use of, and/or processing of coded personal health information and other personally identifiable information will be obtained in accordance with the applicable laws, such as US Health Insurance Portability and Accountability Act regulations, state data privacy laws, the EU General Data Protection Regulation (GDPR), or other applicable national or local data usage and protection laws.

In accordance with applicable local laws and with the subject's express and optional consent, any remaining biological samples taken for the purposes of the study, as well as any voluntary samples, may be used by Ultragenyx for additional research and development in accordance with the subject's express consent. Any such consent will be optional and not a requirement to study participation.

Before conducting any study procedures, the Investigator must obtain written informed consent for or from each potential subject. Part of obtaining that written informed consent requires the Investigator to fully explain to each potential subject the methods, objectives, requirements, and potential risks of the study and to answer any inquiries. Further, the Investigator must explain to each potential subject that the subject is completely free to refuse to enter the study or to withdraw from the study at any time without either decision impacting his/her care. The Investigator or a qualified designee must be available to answer each subject's questions throughout the study, and all of the subject's questions must be answered to the subject's satisfaction.

Pediatric subjects or adult subjects with cognitive limitations will provide written assent (if possible) and a legally authorized representative (parent or legal guardian) must provide written informed consent for such subjects. If, over the course of the study, a pediatric subject becomes old enough for a subsequent assent or reaches the legal age of majority, he/she must sign the then-age-appropriate assent or consent as the case may be.

Subjects will be given a copy of the signed ICF and/or assent and will be provided any new information during the course of the study that might affect their continued participation in the study. If the protocol is amended and the ICF is revised, each subject will be required to provide written informed consent again using the revised ICF. The signed ICF or assent will remain in each subject's study file and must be available to the study monitor(s) at all times.

Data Protection, Anonymization, and Security

Any study data transferred to Ultragenyx will be coded and will not contain names or any other information that would make the subject identifiable.

Study information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from Ultragenyx.

The subject must be informed of his/her rights under applicable data protection laws regarding how his/her personal information is being treated. Additionally, the subject must be informed, and where possible, be provided with the details and references, that certain persons or entities may have access to his/her medical records for various, legitimate reasons and purposes, including Ultragenyx's auditors or other authorized personnel appointed by Ultragenyx may examine his/her medical records, appropriate IRB/IEC members, and by inspectors from regulatory authorities.

The subject must also be provided with the applicable contact information for Ultragenyx, site, and/or the country's/regional's Data Protection Authority in order to inquire about their rights, raise concerns, or issue complaints regarding the treatment of his/her personal information by Ultragenyx.

Ultragenyx complies with applicable data protection laws and treats personal information in accordance with standard security measures. Ultragenyx has implemented policies and procedures to prevent security issues, including data breaches, and avoid/minimize any negative consequences. All computerized systems to be used during the study have been selected following a strict process that encompassed a System Level Risk Assessment, including system identification, GxP, GDPR, Sarbanes and Oxley Act, and 21 CFR Part 11 applicability. As far GDPR compliance is concerned, a Data Classification Assessment which includes the Data Privacy Impact Assessment (DPIA) was also conducted on all systems, and their results are memorialized in DPIA assessments stored in the Ultragenyx Document Management System. Ultragenyx imposes the same principles to all vendors supporting the study, which translates into (i) selecting such vendors and their systems against equally stringent security protocols and standards; (ii) ensuring that they comply with applicable data protection laws, and (iii) agreeing to specific data protection terms that include, among others, a data breach response plan and an obligation to collaborate with Ultragenyx in such circumstances timely. These measures exist so Ultragenyx can mitigate any security occurrence and meet their obligations under applicable data protection laws. Therefore, in case of a data breach, Article 33 GDPR will be followed. Additionally, in order to maintain/restore a GxP-compliant environment, an audit will be conducted, and the necessary remedial actions implemented in a timely fashion (including, but not limited to, termination of any relevant third party from the research) to guarantee the integrity of the concerned data and avoid any future repetition.

Protocol Deviations

A protocol deviation is any instance of protocol noncompliance, either at study entry or during the study conduct. A major protocol deviation is defined by Ultragenyx as a having a significant impact on a clinical study data and subject rights, safety, or welfare. All other protocol deviations are considered minor protocol deviations.

Ultragenyx does not issue protocol deviation waivers. The Investigator must inform Ultragenyx of all protocol deviations in a timely manner; provided, however, that Ultragenyx be informed of all major Protocol deviations immediately of becoming aware of such deviations.

Future Use of Stored Study Samples

With the subject's written consent, Ultragenyx will use remaining protocol-driven samples and/or will collect optional, non-protocol-required biologic samples to be used for additional future research. These samples will be de-identified and may be stored for up to 20 years from the end of the study, or shorter as required by local law.

Investigators and Study Administrative Structure

Prior to the Investigator beginning performance of the study, Ultragenyx must be in receipt of all essential documents, including, among others, a signed Form FDA 1572 or equivalent signed by the Investigator and any sub-investigators, and an appropriate Financial Disclosure Form from each.

A Coordinating Investigator will be identified for multicenter studies. The Coordinating Investigator will be selected on the basis of active participation in the study, thorough knowledge of the therapeutic area being studied, and the ability to interpret data. The Coordinating Investigator will read and sign the Clinical Study Report.

Investigational Product Accountability

The Investigator must be thoroughly familiar with the appropriate administration and potential risks of administration of the IP, as described in this protocol and the Investigator Brochure (IB), prior to initiation of the study.

IP delivered to the study site and/or Investigator must be stored in a secure limited access location at controlled temperature as described in the IB and product packaging. Any such location must be available for inspection by the study monitor or auditors for inspection at any time during the study. If subjects take IP offsite, they will be given instructions on the proper storage of IP when initially dispensed and reminded of storage requirements at all subsequent visits.

An IP accountability record must be maintained for all IP received, dispensed, returned, destroyed, and/or lost during the study. This record must be kept current and made available to the study monitor or auditors for inspection. At the completion of the study, all unused IP must be returned to Ultragenyx and/or its designee, unless other instructions have been provided for final disposition of the IP.

Data Handling and Record Keeping

Case Report Forms and Source Documents

The Investigator is required to initiate and maintain, for each subject, an adequate and accurate case history that records all observations and other data related to the study for that subject. Data must be recorded on Case Report Forms (CRFs), electronic or otherwise, in accordance with the Clinical Trial Agreement executed between Ultragenyx and the study site and/or Investigator. Recorded data is subject to Ultragenyx verification. All information recorded on CRFs must be consistent with the subject's source documentation. For electronic CRFs, a validated Electronic Data Capture (EDC) system will be used for entry of the data.

Data entry and data corrections will be made only by Ultragenyx-authorized users and will be captured in an electronic audit trail. All data entered in to the CRF must be verifiable; therefore, CRFs will be routinely checked for accuracy, completeness, and clarity and will be cross-checked for consistency with source documents, including laboratory test reports and other subject records by Ultragenyx or its designee. The Investigator must allow Ultragenyx or its designee direct access to all source documents.

Data Quality Assurance

Monitoring and auditing procedures will be implemented to ensure compliance with Applicable Study Conduct Obligations. Ultragenyx's designated representative (ie, the site monitor) will contact the Investigator and conduct regular visits to the study site. The site monitor will also be responsible for confirming adherence to the study protocol, inspecting CRFs and source documents, and ensuring the integrity of the data. Instances of missing or uninterpretable data will be resolved in coordination with the Investigator.

The site monitor will also investigate any questions concerning adherence to regulatory requirements. Any administrative concerns will be clarified and followed. The site monitor will maintain contact through frequent direct communications with the study site by e-mail, telephone, facsimile, and/or mail. The Investigator and all other personnel supporting the study (ie, Project Personnel) must agree to cooperate fully with the site monitor and will work in good faith with the site monitor to resolve all questions raised and all identified issues.

Record Retention

For study monitoring, audit, or inspection, the IRB/IEC and Ultragenyx or its designees have the right to access all CRFs, source documents, and other study documentation. The Investigator or study site will retain such documents from the start of the study to at least 25 years after the close of the study, guarantee access to these documents and cooperate with and support such audits and inspections.

Publication Policy

The Investigator and/or study site must submit any publication or presentation about the study to Ultragenyx prior to making it public in accordance with the process in the Clinical Trial Agreement executed between Ultragenyx and the study site and/or Investigator.

Registration of Study and Disclosure of Results

Ultragenyx will register the study and post results regardless of outcome on a publicly accessible website in accordance with the applicable laws and regulations.

Budgeting and Insurance

The budget and insurance for this clinical study will be addressed in the Clinical Trial Agreement between Ultragenyx and the study site and/or Investigator executed between Ultragenyx and the study site and/or Investigator.

APPENDIX 6. PROTOCOL AMENDMENT HISTORY

Table 16: Protocol Amendment History

Document	Date of Issue	Overall Rationale for the Amendment
Amendment 2.3	03 January 2022	To clarify the timing of assessments, use of premedications, and procedures for glucose monitoring
Amendment 2.1	01 October 2021	This protocol amendment was submitted to FDA on 19 October 2021 and withdrawn on 01 December 2021. Changes included in this withdrawn protocol amendment are not reflected in Table 17.
Amendment 1	24 March 2021	To clarify and align with recommendations from regulatory authorities
Original Protocol	20 January 2021	Not applicable

Table 17: Summary of Protocol Amendment Changes

Amendment 2.3 / 03 January 2022 / Substantial		
Section # and Name	**Description of Change**	**Brief Rationale**
Schedule of Event Table 6	The liver MRI, MRS, and Fibroscan/ultrasound elastrography occurring during screening was moved from the Stablization Visit (STV) to the Initial Screening Visit (ISV).	Hepatic imaging will help determine if subjects meet eligibility criteria, such as exclusion based on the presence of cirrhosis.
Schedule of Event Table 6, Section 5.4.2 Potential Risks for Premedications, Section 7.1 Study Design, Section 9 Interventions, and Section 9.1.1 Premedications	At the discretion of the Investigator, with input from Ultragenyx and the Data Monitoring Committee (DMC), and based on the emerging safety profile of UX053, additional premedications and rescue medications may be used to reduce the risk and severity of immune reactions in Repeat Dose (RD) cohorts. Premedication and rescue medication selection will be based on the nature of the reactions being observed and the risks of such medications in patients with GSD III.	Acetaminophen, ibuprofen, cetirizine, famotidine, and dexamethasone may not be sufficient to prevent immune-mediated reactions to UX053. If infusion-related reactions are observed, other medications may be used to minimize risk to study subjects.

Amendment 2.3 / 03 January 2022 / Substantial		
Section # and Name	**Description of Change**	**Brief Rationale**
Schedule of Events Table 4 and Table 6	A row was added to both Schedule of Events tables to indicate that all visits after Day 7 in the SAD Period and Week 1 in the RD Period may occur within a window of ± 2 days. In line with this change, in Table 8, the window of ± 24 hours for Day 14, 21, and 28 for pharmacokinetic sampling is now ± 48 hours. If all assessments for a given visit occur within the visit window, it will not constitute a protocol deviation. Some assessments may occur within a week prior to dosing (footnote c of Table 4 and footnote d in Table 6).	A visit window was established to reduce patient burden.
Schedule of Events Table 4 and Table 6, Section 7.1, and Section 10.5.3 Continuous Glucose Monitoring	The following text was added: "At the Screening Visit for the SAD cohort and ISV for the RD cohort, all subjects will receive continuous glucose monitoring (CGM) and handheld dynamometry (HHG) devices and will be trained on the proper use of these devices. Subjects will also be trained on completion of the nutrition diary. When CGM data is being collected, all subjects will connect their CGM and HHG devices to a study-provided laptop weekly and upload CGM and HHG data. At remote visits, in conjunction with assessments performed by a Home Health nurse, the site will contact subjects by telephone or videoconference to confirm the CGM device is properly in use and data was uploaded; answer any questions regarding or help troubleshoot (if necessary) CGM device use; and review CGM data, HHG data (when applicable), and nutrition diary entries; these telephone calls will occur as needed for the SAD Period between Day 28 and Day 90/EOS I Visits and for the RD Period between the Week 12 and 24 Visit. At in-clinic visits, a conversation between the site and the subject should occur to ensure CGM compliance and troubleshoot if needed." Related to this addition, the following text was removed from the schedule of events tables "The site should record the handheld glucometer data in the CRF at the next site visit."	The site no longer needs to record HHG data in the Case Report Form (CRF), as subjects are asked to upload both CGM and HHG data on a weekly basis when CGM is being collected. This additional text also clarifies how CGM and HHG data will be collected.
Schedule of Events Table 4 and Table 6, and Section 10.5.5 Limit Dextrins	Hexose tetrasaccharide (Hex_4) nomenclature is changed to glucose tetrasaccharide (Glc_4), which is a more specific description of this analyte.	Hexose tetrasaccharide (Hex_4) includes glucose tetrasaccharide and maltotetraose, but only glucose tetrasaccharide will be measured.

Amendment 2.3 / 03 January 2022 / Substantial		
Section # and Name	**Description of Change**	**Brief Rationale**
Schedule of Events Table 4 and Table 6, and Appendix 3 Laboratory Assessments	Appendix 3 was updated to include all laboratory assessments instead of only safety laboratory assessments. Related to this change, creatine kinase was removed from the chemistry panel in Appendix 3, as it is already collected under Skeletal Muscle & Strength Assessments, noted in Table 4 and Table 6.	To further clarify all labs being measured, and to clarify that creatine kinase is not measured twice for a given visit.
Schedule of Events Table 4	A footnote was added to the SAD Period Schedule of Events Table 4 to indicate that a complete physical exam is required at the Screening, Baseline, and End of Study/Early Termination Visits. A targeted physical exam may be performed at the Day 1 Visit.	To clarify requirements for physical exams.
Schedule of Events Table 4	The Patient-Reported Outcomes Measurement Information System (PROMIS) questionnaires, Short Form Health Survey 36 Version 2 (SF-36v2), and GNE Myopathy Functional Activities Scale (GNEM-FAS) were removed from the Screening Visit during the SAD Period.	During the SAD Period, the PROMIS questionnaires, SF-36v2, and GNEM-FAS do not need to be assessed at the Screening Visit, as they are assessed at the Baseline Visit, which occurs shortly after the Screening Visit.
Schedule of Events Table 4 and Table 6	Serum B-type natriuretic peptide (BNP) was updated to plasma BNP.	Plasma will be used for the assessment of BNP.
Section 10.4.1.1 Liver Magnetic Resonance Spectroscopy and Section 0 Calf Magnetic Resonance Spectroscopy	Magnetic resonance spectroscopy (MRS) is no longer limited to ^{13}C MRS.	Imaging methodology was expanded based on site feasibility. Broadening the methodology will increase the number of sites capable and therefore provide more meaningful imaging results.
Schedule of Events Table 4 and Table 6; Section 0 GDE Enzyme Activity	Peripheral blood mononuclear cell (PBMC) collection for the assessment of glycogen debranching enzyme (GDE) enzyme activity will no longer be collected.	Nonclinical studies revealed that this assessment will not reliably predict GDE activity, and is therefore no longer necessary.
Schedule of Events Table 4 and Table 6;Section 0 Exploratory Future Use Blood Cell Pellet, Serum, and Plasma Collection	Blood cell pellets for future use will now be collected instead of whole blood samples for limit dextrins. Reflecting this change, “whole blood limit dextrins” was removed from the “whole blood limit dextrins & plasma Glc4” row; and a new row for “blood cell pellet for future use” was added. Though there is no change to the sample collection schedule, the analysis of the blood samples has changed.	Nonclinical studies revealed that UX053 treatment is unlikely to impact glycogen content in circulating blood cells. Instead, blood cell pellets will be used to measure GDE production in a more sensitive manner.

Amendment 2.3 / 03 January 2022 / Substantial		
Section # and Name	**Description of Change**	**Brief Rationale**
Amendment 1 / 24 March 2021 / Substantial		
Section # Name	**Description of Change**	**Brief Rationale**
Title Page	The D and EudraCT number was added.	This information was not available for the finalization of the original protocol.
Title Page	The contact information for the Medical Monitor was updated to include a telephone number in addition to an email address. The address was removed.	A telephone number and email address is the more appropriate method of contact.
Schedule of Events Table 4 and Table 6, Section 7.1 Study Design, Section 9.7 Treatment Compliance, Section 10.4.4.4 Nutrition Diary, and Section 11.8 Nutrition Diary	The term “diet diary” was changed to “nutrition diary” throughout the protocol.	The name of the assessment was changed to more accurately reflect the data being captured.
Schedule of Events Table 4 and Table 6, Section 10.1.5 Safety Laboratory Tests, and Section 10.1.5 Safety Laboratory Tests	The footnote referring to urine collections was updated to clarify that all efforts should be made for urine collections to be consistently taken from the first morning void. Relevant text was also updated in Section 10.1.5 Safety Laboratory Tests and Section 10.5.5 Limit Dextrins, Including Hexose Tetrasaccharide.	To clarify
Schedule of Events Table 6	Vital signs was added as an assessment at the home health Week 1 visit during the RD Period.	Vital signs assessment at this visit was accidentally omitted from the original protocol.

Amendment 2.3 / 03 January 2022 / Substantial		
Section # and Name	**Description of Change**	**Brief Rationale**
Schedule of Events Table 6	Additional home health visits at Weeks 3, 5, and 7 in the Repeat Dose (RD) Period were added to the RD Schedule of Events Table 6 and relevant footnotes. Assessments at these home health visits include: vital signs, adverse events (AEs), concomitant medications, hematology, chemistry, urinalysis, serum C-reactive protein (CRP), nutrition diary, and continuous glucose monitoring.	To ensure subject safety per recommendation from regulatory authorities
Schedule of Events Table 6	Sample collection for anti-drug antibody (ADA) analysis was added at Weeks 2 and 6 in the RD Period. With this update, ADA collection occurs at Baseline and Weeks 2, 4, 6, 8, 10, 24, and 48 (End of Study Visit).	Updated per recommendation from regulatory authorities
Schedule of Events Table 6	Footnote K and the corresponding assessments in the Schedule of Events Table 6 were updated to indicate that All imaging assessments (liver MRI, liver MRS, Fibroscan or ultrasound elastography, and calf MRS) can be conducted within 1 week prior to the corresponding visit.	To minimize subject burden by having the option to spread assessments across multiple days
Schedule of Vital Sign Assessments Table 7	An optional vital sign assessment was added at the time of discharge.	Subjects are monitored in the clinic for 24 hours from the end of study drug infusion. This addition of this optional vital sign assessment will capture any vital signs assessed just prior to discharge.
Section 5.5 Risk Minimization and Section 7.1 Study Design	The Single Ascending Dose (SAD) cohort data review was updated from an internal review to a review by the independent Data Monitoring Committee (DMC). Additional text was added to clarify that all cumulative data from prior cohorts, when applicable, will be reviewed in addition to data from within a cohort before subsequent dosing cohorts may begin.	Updated per recommendation by regulatory authorities

Amendment 2.3 / 03 January 2022 / Substantial		
Section # and Name	**Description of Change**	**Brief Rationale**
Section 5.5 Risk Minimization, Section 7.1 Study Design, and Section 7.1.5 Dose Rationale	The interval between dosing of individual subjects within a given SAD cohort was updated from a minimum of 48 hours to a minimum of 72 hours.	• Updated to ensure review of post-treatment laboratory assessments from each subject prior to proceeding with the next subject
Schedule of Events Table 4 and Table 6 and Section 10.1.1 Coronavirus Disease 2019 (COVID-19) Testing	Coronavirus 2019 (COVID-19) testing by real time – polymerase chain reaction (RT-PCR) was added to the Initial Screening Visit.	Updated per recommendation by regulatory authorities
Schedule of Events Table 4 and Table 6 and Section 10.5.4 Hemoglobin A1C	Hemoglobin A1C was added to the Initial Screening Visit for both the SAD and RD Periods, as well as the Week 12 and Week 48/End of Study (EOS) Visit for the RD Period.	To ensure subject safety per recommendation by regulatory authorities
Section 7.1 Study Design	Criteria for discharge 24 hours after the end of study drug administration were added.	To ensure subject safety per recommendation by regulatory authorities
Section 7.1 Study Design	The following statement “Mean weekly protein intake < 20% of total calorie intake and mean weekly carbohydrate intake ≥ 60% of total calorie intake for subjects will be considered protocol deviations” was amended to include “notwithstanding temporary changes to diet due to illness.”	To accommodate subjects who may experience a minor viral illness that impacts food intake for a few days
Schedule of Events Table 6 and Section 7.1 Study Design	Additional text was added to clarify the process in which a subject moves through the screening visits (Initial Screening Visit, Nutrition Optimization Visits [if needed], and Stabilization Visit) in the RD Period. Related text in the footnotes of the Schedule of Events Table 6 was also updated for clarity.	To clarify the sequence of events for the screening visits in the RD Period

Amendment 2.3 / 03 January 2022 / Substantial		
Section # and Name	**Description of Change**	**Brief Rationale**
Section 7.1 Study Design and Section 9.1.4 Guidance on Communication and Seeking Immediate Medical Attention	Section 9.1.4 Guidance on Communication and Seeking Immediate Medical Attention was added. This section provides guidance on the encouraged communication between subject, primary care provider, and Investigator. This section also provides guidance to be provided to subjects on signs and symptoms to be vigilant for and how to proceed in the event that these signs and symptoms manifest. This section is referenced in Section 7.1.	To ensure subject safety per recommendation from regulatory authorities
Section 5.5 Risk Minimization, Section 7.1 Study Design, Section 9.1.6 Coronavirus Disease 2019 (COVID-19) Vaccination, and Section 0 Exclusion Criteria	Section 9.1.6 Coronavirus Disease 2019 (COVID-19) Vaccination was added to clarify the ability and timing for subjects to receive a COVID-19 vaccine. COVID-19 vaccination is allowed during study participation. COVID-19 vaccination should not be administered within 48 hours after the most recent administration of UX053 and until safety labs obtained have been reviewed and the subject has been assessed for any possible hypersensitivity reaction to the most recent dose of UX053. Consultation with an allergist/immunologist is recommended prior to a COVID-19 vaccination if a subject has an immediate or severe hypersensitivity reaction to UX053 (Banerji et al., 2020). A reference to this additional text was added to Section 7.1 Study Design. Related to this added section, the following exclusion criterion was added: “Receipt of only 1 of 2 planned doses of a coronavirus disease 2019 (COVID-19) vaccine. Subjects who have not received a COVID-19 vaccine, and those who have completed COVID-19 vaccination are eligible.” Also related to this section, measures taken to minimize risk of COVID-19 exposure were added to Section 5.5 Risk Minimization.	Subjects who develop an immediate or severe hypersensitivity reaction to UX053 are at risk for having a similar reaction to an mRNA based COVID-19 vaccine due to the similarity of the LNP formulations. Based on nonclinical studies, hypersensitivity reactions to UX053 are expected to occur within minutes to hours following drug administration. Therefore, subject safety should be assessed prior to receipt of a COVID-19 vaccine to ensure subject safety. AEs are more likely to occur after the second dose of mRNA-based COVID-19 vaccines. Preventing enrollment of subjects who have not completed a planned second dose of a COVID-19 vaccination ensures subject safety. Subjects who have received only 1 of a planned 2-shot vaccination series should complete the vaccination series prior to screening.

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Section # and Name	**Description of Change**	**Brief Rationale**
Section 5.4.2 Potential Risks for Premedication, Section 7.1 Study Design, Section 7.1.5 Dose Rationale, Section 8.3.1 Discontinuation of Intervention, Subject, or Study, Section 8.3.2 Subject-level Redosing and Stopping Criteria, Section 9.1.1 Premedications, Section 9.1.3 Clinical Observation and Supportive Care, and Section 9.5 Rescue Medications	Section 8.3.2 Subject-level Redosing and Stopping Criteria was added; a reference to this section was added in various sections of the protocol, as relevant. The following information was provided: requirements for redosing study drug, criteria that exclude subjects from redosing (eg, developing new or worsening symptoms of liver disease, receipt of blood products for treatment of consumptive coagulopathy, and specific elevations in ALT levels), and action required in the event that an IAR occurs, as specified by Common Terminology Criteria for Adverse Events (CTCAE) severity. Additionally, text in Section 7.1 Study Design was updated to reflect that Ultragenyx will notify the DMC immediately if any subject experiences an event that satisfies subject-level or study-level stopping criteria.	To ensure subject safety per recommendation from regulatory authorities
Section 7.1 Study Design, Section 7.1.4 Dose Rationale, Section 8.3.2 Subject-level Redosing and Stopping Criteria, Section 9.1.1 Premedications, and Section 9.1.3 Clinical Observation and Supportive Care	If dexamethasone (or equivalent) is used as a rescue medication, premedication with dexamethasone (or equivalent) should be considered for all subjects' subsequent infusions. Previous text indicated that dexamethasone (or equivalent) should be considered as a premedication only for subjects who required dexamethasone (or equivalent) as a rescue medication.	To ensure subject safety per recommendation from regulatory authorities
Section 7.1.3 Rationale for Study Design	Text was added to expand upon the rationale for the use of a placebo control arm.	Updated per recommendation from regulatory authorities
Section 8.1 Inclusion Criteria	An additional inclusion criterion was added to ensure enrolled subjects have clinically significant disease manifestations that are likely to be improved by UX053 treatment.	Updated per recommendation from regulatory authorities
Section 8.1 Inclusion Criteria	The following inclusion criterion was added "ALT ≤ 5x ULN during the 3 months prior to the Baseline Visit."	Updated to facilitate review of potential drug-induced liver injury (DILI) signals

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Section # and Name	**Description of Change**	**Brief Rationale**
Section 8.2 Exclusion Criteria	CCI [redacted]	• CCI [redacted]
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "Severe renal impairment defined as a defined as a glomerular filtration rate (GFR) ≤ 29 mL/min (Levey et al., 2005)."	Subjects with severe renal impairment may be more vulnerable to fluid shifts that could occur in the setting of a hypersensitivity reaction
Section 8.2 Exclusion Criteria	Resistant hypertension was added to the exclusion criterion for cardiac diseases: "Significant cardiac disease, including heart failure with New York Heart Association (NYHA) Function Capacity III or IV or Objective Assessment C or D, unstable angina, or ejection fraction (EF) < 35%, or uncontrolled arrhythmia or resistant hypertension (Carey et al., 2018). Asymptomatic cardiomyopathy and left ventricular hypertrophy (LVH) are allowed."	Patients with persistent hypertension are at high risk for adverse cardiovascular events, despite multiple medications; such patients could affect interpretation of safety data
Section 8.2 Exclusion Criteria	Poorly controlled diabetes and its definitions were added to the exclusion criteria	To ensure subject safety and data interpretability
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "Poorly controlled hypothyroidism, based on the judgement of the Investigator or Ultragenyx, whichever is most conservative"	To ensure subject safety and data interpretability
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "History of chronic coagulopathy, thrombophilia, or disorder of complement activation"	To ensure subject safety and safety data interpretability

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Section # and Name	**Description of Change**	**Brief Rationale**
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "Use of concomitant medications that alter PT/INR, including warfarin and direct oral anticoagulants (eg, rivaroxaban, apixaban, and edoxaban). Patients who receive medications that affect platelet function, such as aspirin or clopidogrel, are allowed unless they have comorbidities that in the judgment of the Investigator place them at undue risk to participate in the study."	To ensure subject safety and interpretation of potential DILI signals
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "Current treatment with long-term immunosuppressive medications. This includes subjects with autoimmune conditions managed with immunosuppressive medications and solid organ transplant recipients"	To ensure subject safety per recommendation from regulatory authorities
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "Active tuberculosis requiring treatment in the past 3 years"	To ensure subject safety per recommendation from regulatory authorities
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "Symptomatic COVID-19 infection"	To ensure subject safety per recommendation from regulatory authorities
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "History of active alcohol and/or drug abuse that in the Investigator's assessment would impair the subject's ability to comply with the protocol"	To ensure subject safety per recommendation from regulatory authorities
Section 8.2 Exclusion Criteria	The following exclusion criterion was added: "Females of childbearing potential with hepatocellular adenoma who are unwilling to use nonhormonal contraception."	To ensure subject safety per recommendations from regulatory authorities

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Section # and Name	**Description of Change**	**Brief Rationale**
Section 8.3.1 Discontinuation of Intervention, Subject, or Study	Ultragenyx will no longer reserve the right to discontinue subjects or the study for administrative reasons. Subjects will only be removed from the study due to withdrawal of consent. Dosing may be discontinued to protect subject's safety, but subjects will remain in the study for safety monitoring purposes, unless continued participation in the study poses a risk to the subject due to a concurrent medical condition or illness.	Updated per recommendation from regulatory authorities
Section 8.3.1 Discontinuation of Intervention, Subject, or Study	The following text, which is already included as a footnote in the Schedule of Events Tables (Table 4 and Table 6), was added to Section 8.3.1 Discontinuation of Intervention, Subject, or Study: "In the event a subject has an early termination (ET), all efforts will be made to monitor the subject through the end of the study. At minimum, a safety follow-up phone call will occur within the 4 weeks following the subject's last treatment."	To ensure subject safety per recommendation from regulatory authorities
Section 8.3.3 Study Stopping Criteria	The following study stopping criterion was added: "Anaphylaxis, as defined by the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network (NIAID FAAN) criteria (Table 11), pending a DMC safety review"	To ensure subject safety per recommendation from regulatory authorities
Section 8.3.3 Study Stopping Criteria	The following study stopping criterion was added: "Any treatment-emergent adverse event (TEAE) with a severity ≥ Grade 3 (CTCAE version 5.0) affecting the cardiopulmonary, renal, or neurological systems, regardless of relationship to study drug, pending a DMC safety review"	To ensure subject safety per recommendation from regulatory authorities

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Section # and Name	**Description of Change**	**Brief Rationale**
Section 8.3.3 Study Stopping Criteria	The following study stopping criterion was modified from "Increases in serum concentrations of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2x from Baseline and > 3x the upper limit of normal (ULN), accompanied by increases in serum concentrations of total bilirubin > 2x ULN or international normalized ratio > 1.5, without any other underlying cause that may account for such changes" to "Increases in ALT or AST > 2x from Baseline and > 3x the ULN, accompanied by total bilirubin > 2x ULN or INR > 1.5, without findings of cholestasis (defined as serum ALP activity < 2x ULN) and in the absence of a plausible alternate explanation."	To ensure subject safety per recommendation from regulatory authorities
Section 8.3.3 Study Stopping Criteria	The following study stopping criterion was added: "3 subjects develop platelet counts < 50,000 per mm^3 (or thrombocytopenia ≥ Grade 3 in severity)"	To ensure subject safety per recommendation from regulatory authorities
Section 8.3.3 Study Stopping Criteria	The following study stopping criterion was added: "3 subjects develop INR > 1.5 (or INR increase ≥ Grade 2 in severity)"	To ensure subject safety per recommendation from regulatory authorities
Section 8.3.3 Study Stopping Criteria	The following text was added: "Regulatory authorities will be notified immediately if the study stopping criteria are met and study enrollment or dosing is halted."	Updated per recommendation from regulatory authorities
Section 8.3.4 Subject Replacement and Section 11.1 Sample Size Determination	Text was added to indicate that subjects will not be replaced if they have received any study drug.	Updated per recommendation from regulatory authorities
Section 9.3 Nutrition Management and Section 9.7 Treatment Compliance	All instances of "Nutrition Guide" were changed to "Study Reference Manual."	Nutritional guidance will be available in the Study Reference Manual.
Section 10.1.2 Vital Signs	Oxygen saturation was added to the list of vital sign assessments.	To ensure subject safety

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Section # and Name	**Description of Change**	**Brief Rationale**
Section 10.1.3 Electrocardiogram	RR was added to the list of measurements calculated for the electrocardiogram assessement.	To ensure subject safety
Section 0	In addition to glycogen content, muscle fat fraction in the calf will also be assessed during the calf ^{13}C Magnetic Resonance Spectroscopy (MRS) assessment.	Fatty degeneration of muscle should also be assessed, as it can affect the ^{13}C MRS signal for glycogen quantitation in muscle.
Section 10.5.6 *AGL* Gene Sequencing	Text was added to clarify that blood collected for *AGL* mutation analysis will be analyzed by a central laboratory.	To clarify the method of analysis
Appendix 1 Safety Definitions and Assessments	Text was added to define a TEAE.	Updated per recommendation from regulatory authorities
Appendix 1 Safety Definitions	Previously, the severity of all AEs were to be graded using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, published on November 27, 2017. Text was added to reflect that the AE Cytokine Release Syndrome (CRS) will be graded by the American Society for Transplantation and Cellular Therapy (ASTCT) Consensus Grading for CRS (Lee et al., 2019). The grading scale is provided in Table 13.	Updated per recommendation from regulatory authorities
Appendix 2 Adverse Event Collection: Eliciting, Recording, and Reporting	The end of the safety reporting period was updated to 28 days following the final dose of study drug or the EOS I (SAD Period) and EOS II (RD Period) final study visit, whichever occurs later. Additionally, suspected related SAEs can be reported at any time following the EOS I/II visit.	To ensure subject safety
Appendix 3 Safety Laboratory Assessments	Magnesium and bicarbonate were added to the list of assessments included in the standard chemistry panel.	Magnesium and bicarbonate were accidentally omitted from the standard chemistry panel in the original protocol.

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Section # and Name	**Description of Change**	**Brief Rationale**
Appendix 4 Contraception Guidance	The text “plus 90 days for a full sperm cycle” was removed from the following statement: “The time period required is generally determined by the pharmacokinetic properties of the study product and non-clinical reproductive toxicity data, plus 90 days for a full sperm cycle.”	Guidance regarding the time period for contraception use in males to prevent pregnancy is 1 month after the last dose of UX053, as discussed in the Informed Consent Form

STATEMENT OF COMPLIANCE

Protocol Title: A Phase 1/2 First-in-human, Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses and Repeat Doses of UX053 in Patients with GSD III

Protocol Number: UX053-CL101

Amendment Number and Date: Amendment 3.1 (US-specific) 14 July 2022

INVESTIGATOR SIGNATURE:

I have read the Protocol and agree to conduct the study as detailed in this protocol and in compliance with the Declaration of Helsinki, Good Clinical Practice (GCP), and all applicable regulatory requirements and guidelines.

Investigator Signature Date

Printed Name: _____

SPONSOR SIGNATURE:

As the Sponsor representative, I confirm that Ultragenyx Pharmaceutical Inc will comply with all Sponsor obligations as detailed in this protocol and in compliance with the Declaration of Helsinki, Good Clinical Practices (GCP), and all applicable regulation requirements and guidelines. I will ensure that the Investigator is informed of all relevant information that becomes available during the conduct of this study.

PPD Date

Ultragenyx Pharmaceutical Inc.