Official Title: A Phase 1/2, Baseline-controlled, Non-randomized, Open-label, Single-ascending Dose Study of a Novel Adeno-associated Viral Vector (FLT190) in Patients with Fabry Disease (FLT190-01)

NCT Number: NCT04040049

Document Date: SAP, Version 1.0, 23 April 2024

FREELINE

STATISTICAL ANALYSIS PLAN

Trial Sponsor:	Freeline Therapeutics Ltd. Sycamore House Gunnels Wood Road Stevenage SG1 2BP United Kingdom
Protocol Number:	FLT190-01 and FLT190-02
IND Number:	024628
Investigational Drug:	FLT190-01: FLT190 (AAV2/S3-FRE1-GLA-co) FLT190-02: no dosing
Indication:	Fabry disease
Dosage Form/Strength:	FLT190-01: Cohort 1: 0.75 x 10^{12} vector genomes [vg] per kilogram (kg) of body weight • Cohort 2: 1.5 x 10^{12} vg/kg • Cohort 3: 4.5 x 10^{12} vg/kg • Cohort 4: 1.3 x 10^{13} vg/kg

FLT190-01 Protocol Title: A Phase 1/2, Baseline-controlled, Non-randomized, Open-label, Single-ascending Dose Study of a Novel Adeno-associated Viral Vector (FLT190) in Patients with Fabry Disease

FLT190-02 Protocol Title: A Multicenter, Long-term, Follow-up Study to Investigate the Safety and Durability of Response Following Dosing of an Adeno-associated Viral Vector (FLT190) in Subjects with Fabry Disease

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CHANGE LOG FOR CHANGES MADE AFTER THE INITIAL APPROVAL

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GLOSSARY OF ABBREVIATIONS

Abbreviation	Term
AAVS3	Adeno-associated virus vector
AE	Adverse event
αGLA	α-galactosidase A
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under curve
ATC	Anatomical Therapeutic Chemical
BMI	Body mass index
BP	Blood Pool
BQL	Quantification limit
CA T1	T1 relaxation time of contrast agent
CWM	Cerebral white matter
CKD-EPI	Chronic kidney disease epidemiology collaboration
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
DBP	Diastolic blood pressure
DLT	Dose-limiting toxicity
DMC	Data monitoring committee
eGFR	Estimated glomerular filtration rate
ECG	Electrocardiogram
ECV	Extracellular volume
EQ-5D-5L	EuroQol 5-Dimension 5-Level
ERT	Enzyme replacement therapy
FACIT	Functional Assessment of Chronic Illness Therapy
FASTEX	Fabry Stabilization Index
FAS	Full analysis set
Gb3	Globotriaosylceramide
GI	Gastrointestinal
GSRS	Gastrointestinal Symptom Rating Scale
HBcAb	Hepatitis B core antibody
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen

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Abbreviation	Term
HBcAb	Hepatitis C antibody
HCV	Heart Chamber Volume
HIV	Human immunodeficiency virus
ICH	International council for harmonization
ICF	Informed consent form
IMP	Investigational medicinal product
INR	International normalized ratio
IV	Intravenous(ly)
KDIGO	Kidney disease: improving global outcomes
LV-RV	Left ventricle-right ventricle
LFT	Liver function test
LysoGb3	Globotriaosylsphingosine
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MSSI	Mainz Severity Score Index
NCI	National cancer institute
NIMP	Non-investigational medicinal product
PCR	Polymerase chain reaction
PCT	Pharmacological chaperone therapy
PKS	Pharmacokinetic set
PR	P wave to R wave interval
PTPs	Previously treated patients
PT	Preferred term
PUPs	Previously untreated patients
QoL	Quality of life
ROI	Region of Interest
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SD	Standard deviation
SFBPI	Short Form Brief Pain Inventory
SOC	System organ class
SOP	Standard operating procedure
TB	Tuberculosis
TEAE	Treatment emergent adverse event
TFL	Table, figure and listing

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Abbreviation	Term
UPCR	Urine protein to creatinine ratio
Vg	Vector genomes
VZV	Varicella zoster virus
WHO	World health organization
WHODD	World Health Organization Drug Dictionary

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1. INTRODUCTION

This Statistical Analysis Plan (SAP) outlines the statistical methods for the display, summary and analysis of data collected within the scope of the FLT190-01 and FLT190-02 study protocols. As with any SAP, the proposed methods and approaches to the data analysis should be deemed as flexible and should allow for changes in the plan to the extent that deviations from the original plan would provide a more reliable and valid analysis of the data. As such, the statistical analysis to a certain degree is iterative since much of the planning is based on assumptions that require verification. The purpose of this plan is to provide general guidelines from which the analysis will proceed. Nevertheless, deviations from these guidelines must be substantiated by sound statistical rationale.

The SAP should be read in conjunction with the study protocol and the Case Report Forms (CRFs). This version of the SAP has been developed using the most recent version of the protocol and the most recent version of the annotated CRFs.

1.1 Responsibilities

Everest Clinical Research will perform the statistical analyses and are responsible for the production and quality control of all tables, listings and figures (TLFs).

1.2 Timing of Analysis

The final study analysis of safety and efficacy is planned in the protocols after all participants complete the final study visit or terminate early from the study.

An interim analysis is planned in the FLT190-01 protocol. But for the purpose of an abbreviated clinical report in this SAP, no interim analysis will be conducted.

Additional descriptive summaries may be performed for specific purposes including: to support regulatory submissions, to conduct new post-hoc data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

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2. STUDY OBJECTIVES, ENDPOINTS AND ESTIMANDS

The study objectives and endpoints listed in the protocol will not be analyzed. The FLT190-01 and FLT190-02 studies were terminated early, and the data will be presented primarily in listings. Summaries of data will not be presented.

Table 2-1 shows the study objectives and endpoints listed in the study protocols.

Table 2-1. Study Objectives, Endpoints and Estimand

Primary Objectives	Primary Endpoints
• To investigate the safety of systemic administration of FLT190 in adult males with Fabry disease (FLT190-01) • To assess the long-term safety of FLT190 in participants with Fabry disease (FLT190-02)	• Safety as assessed by the reporting of AEs
Secondary Objectives	**Secondary Endpoints**

• To investigate endogenous production of α-galactosidase A (αGLA) following systemic administration of FLT190 in adult males with Fabry disease. (FLT190-01 and FLT190-02)
 	
• To investigate the clearance of globotriaosylceramide (Gb3) and lysoGb3 measured in plasma and urine. (FLT190-01 and FLT190-02)
 	
• To establish a baseline for long-term follow up of cellular Gb3 inclusions in skin and renal biopsies. (FLT190-01 and FLT190-02)
 	
• To assess viral shedding in various body fluids after systemic administration of FLT190. (FLT190-01 and FLT190-02)
	• Safety
	  • Safety as assessed by reporting of abnormal or change from baseline findings from safety assessments including, laboratory assessments, vital signs, electrocardiogram (ECG), 24-hour Holter monitor, echocardiogram, cardiac magnetic resonance imaging (MRI), physical examination, and liver ultrasound.
	
	• Efficacy
	  • Change from baseline in Gb3 and globotriaosylsphingosine (lysoGb3) in plasma and urine, up to 38 weeks following systemic administration of FLT190.
	  • Volume fraction of Gb3 inclusions per cell, by renal/skin cell type.
	
	• Immune Response to αGLA
	  • Immune response to the human αGLA transgene product will be assessed by measurement of total and neutralizing antibodies up to Week 38 following systemic administration of FLT190.
	
	• Shedding
	  • Clearance of vector genomes (Vg) in blood, urine, saliva, stool, and semen.

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• To describe the immune responses to the endogenous product of αGLA following systemic administration of FLT190. (FLT190-01 and FLT190-02)	• Pharmacokinetic
	  • Change from baseline in plasma αGLA activity and concentration at Week 12, 24, and 38.
	  • Comparison of overall plasma αGLA activity and concentration AUC from baseline to Week 38.

Exploratory Objectives	**Exploratory Endpoints**

• To investigate the impact of endogenous production of αGLA following a single administration of FLT190 on Fabry disease severity, progression, and the requirement for treatment resumption. (FLT190-01 and FLT190-02)
• To investigate the impact of endogenous production of (leukocyte αGLA levels) (FLT190-01 and FLT190-02)
• To investigate the impact of endogenous production of αGLA on estimated glomerular filtration rate (eGFR) (FLT190-01 and FLT190-02)
• To investigate the impact of endogenous production of αGLA on urinary protein and albumin excretion (FLT190-01 and FLT190-02)
• To investigate the impact of endogenous production of αGLA on cardiac parameters (FLT190-01 and FLT190-02)
• To investigate the impact of endogenous production of αGLA on ischemic/hemorrhagic lesions and regions of chronic white matter hyperintensities within the brain (FLT190-01 and FLT190-02)
	• Efficacy
	  • Change from baseline in leukocyte αGLA activity levels.
	  • Change from baseline in eGFR.
	  • Change from baseline in urinary protein and albumin excretion measured using urine spot analysis
	  • Change from baseline in cardiac parameters measured using MRI at Week 38.
	  • Change from baseline in brain imaging measured using MRI at Week 38.
	  • Change from baseline in Fabry disease severity using the Mainz Severity Score Index (MSSI) and Fabry Stabilization Index (FASTEX).
	  • Need for Fabry treatments (including resumption of enzyme replacement therapy (ERT) and pharmacological chaperone therapy (PCT)) after systemic administration of FLT190.
	  • Change from baseline in symptomatic pain assessed using the Short Form Brief Pain Inventory (SFBPI).
	  • Change from baseline in GI symptoms assessed using the Gastrointestinal Symptom Rating Scale (GSRS).
	  • Change from baseline in fatigue assessed using the Functional Assessment of Chronic Illness Therapy (FACIT) - Fatigue scale.
	  • Change from baseline in blood plasma immune biomarkers.
	• Health-related Quality of Life
	  • Change from baseline in QoL score using the EuroQol 5-Dimension 5-Level (EQ-5D-5L) questionnaire at Week 38.
	• Immune Response to AAVS3
	Capsid will be assessed by measurement of:
	  • Anti-AAVS3 antibodies.
	  • T-cell responses to AAVS3 capsid in peripheral blood mononuclear cells.

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• To investigate the impact of endogenous production of αGLA on quality of life (QoL) (FLT190-01 and FLT190-02)
 	
• To investigate the impact of endogenous production of αGLA on health resource utilization (FLT190-01 and FLT190-02)
 	
• To investigate the impact of endogenous production of αGLA on symptomatic pain
 	
• To investigate the impact of endogenous production of αGLA on gastrointestinal (GI) symptoms
 	
• To investigate the impact of endogenous production of αGLA on fatigue levels
 	
• To investigate the impact of endogenous production of αGLA on plasma immune biomarker response (FLT190-01 and FLT190-02)
 	
• To describe the immune response to the adeno-associated virus vector (AAVS3) capsid proteins following systemic administration of FLT190
	• Health Resource Utilization
	  • Change from baseline in the level of health resource utilized by participants including the following:
	  • Number of Fabry disease related medical appointments.
	  • Number of emergency room visits.
	  • Number of hospitalizations related to Fabry disease.
	  • Length of hospital stay.
	  • Number of days lost from education or work by patients and caregivers.

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3. STUDY DESIGN

3.1 Study Design

3.1.1 Protocol FLT190-01

MARVEL 1 is a Phase 1/2, open label, baseline-controlled, non-randomized, single-ascending dose study of FLT190 in participants with Fabry disease.

Participants had undergone screening assessments for up to 18 weeks prior to Study Day 0 (gene therapy infusion).

Treatment-eligible participants reported to the infusion study site on the day prior to receiving the gene therapy infusion (Day -1), (Note: Day -1 assessments may be conducted as early as Day -3 for logistical reasons). Participants had either attended their local study site if it has been approved for FLT190 infusion, or if not, an alternative infusion study site. On Day 0, FLT190 was administered as a single dose, slow IV infusion into a peripheral vein, and the participant remained in the study center for at least 8 hours following the end of the infusion, until the investigator had deemed the participant as fit to be discharged.

Participants were required to undergo study evaluations at intervals over the 38-week post-treatment period. These had taken place either at the study infusion site or at their local study site. To monitor for shedding of vg sequences, participants were required to provide plasma, saliva, urine, stool, and semen samples until three consecutive samples taken at separate visits (at least 1 week apart) were shown to be clear of vg sequences for each matrix. This was a first-in-human study and as such an ascending-dose design had been implemented to enable dose evaluation in a step-wise manner. The study was conducted in 2 parts (Figure 1):

Part 1

Up to 4 dose cohorts of vector were planned to be tested in a dose escalation scheme in previously treated patients (PTPs, defined as patients who are currently receiving either a licensed ERT or PCT and have been receiving the therapy at a stable dose for at least 12 months prior to FLT190 dosing).

Participants in the USA were only to be enrolled for Cohort 2 and above.

Participants receiving both ERT and PCT were not eligible to participate in the study. In Part 1, eligible patients currently receiving ERT maintained their current treatment until a minimum of 2 consecutive results at least 4 weeks post-dosing showed αGLA expression higher than predose trough αGLA expression, after which ERT could be withdrawn. In Part 1, eligible patients currently receiving PCT had their treatment stopped prior to baseline measurements (Day -1). Based on the half-life of current approved PCT a minimum 7-day washout period between final dosing of PCT and Day -1 was observed. Cohorts were planned to include between two and three PTPs. For each participant, safety outcomes (as determined by adverse event (AE) reporting and laboratory tests) and αGLA expression were evaluated after dosing. Dependent on safety and αGLA expression, a decision was taken on whether to recruit another participant into the cohort or to proceed to the next dose cohort (Protocol Sections 14.3.3 and 14.3.4). Dose escalation was overseen by an independent data monitoring committee (DMC).

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An interim analysis was planned when dose escalation was complete in PTP's in Part 1 and all patients had at least 12 weeks of data following FLT190 administration. The aim of this interim analysis was to inform the recommended dose for a Phase 3 study in PTPs. When the Part 1 dose escalation was complete (and all patients had at least 12 weeks of data following FLT190 administration), dosing in Part 2 was planned to be discussed with the DMC to inform the recommended dose for Part 2 in previously untreated patients (PUPs, defined as patients who had never previously received treatment with ERT or PCT [licensed or investigational]) .

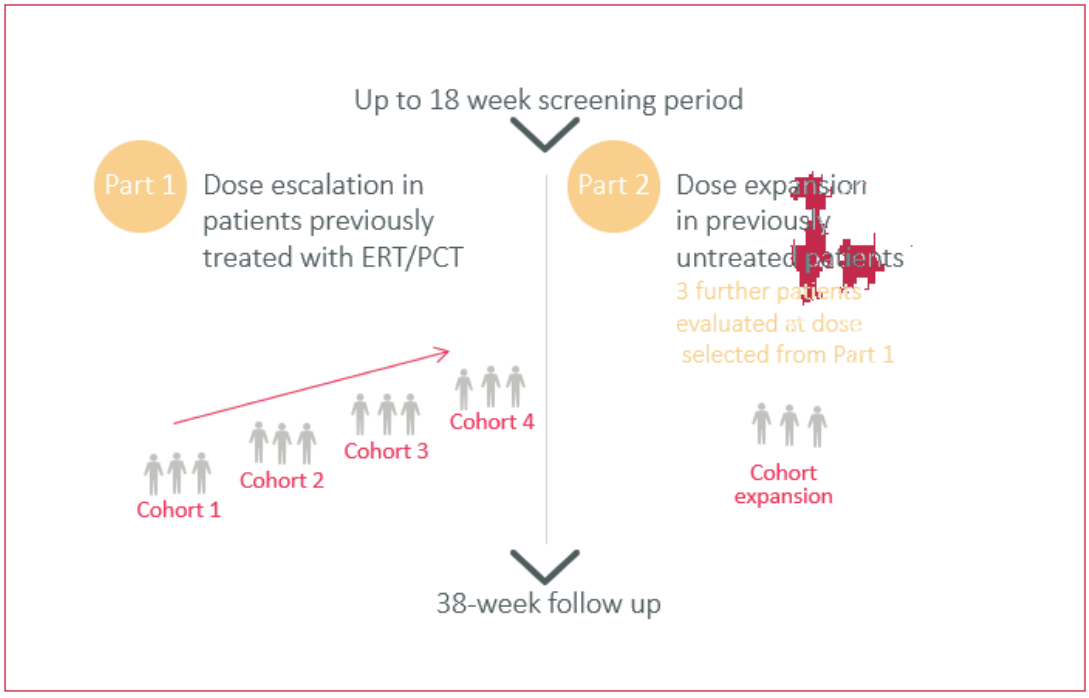
Part 2

A single cohort of three PUPs was planned to be enrolled at the dose level selected from Part 1.

In Fabry disease there is an unmet need in both previously treated and untreated participants. The overall response to FLT190 in terms of tolerability and ability to identify an optimal dose to progress to later studies was not anticipated to be different between these two groups. PTPs are the more prevalent participant group and could demonstrate αGLA expression levels and early tolerability. The PUP group was intended to confirm tolerability and more readily demonstrate modulation of biomarkers. There were likely to be differences in baseline biomarker profiles between the populations e.g. baseline renal Gb3 inclusions were likely to be higher in PUPs. By obtaining data from both populations, the Sponsor would be better able to identify similarities and differences for further study. An incremental risk for PUPs was not anticipated, and they were to be closely monitored in a similar way for any need to initiate alternative treatment.

On completion of the study, participants were to be followed under a separate long-term follow-up protocol for a total of 5 years after dosing.

Figure 1. Study Schematic



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3.1.2 Protocol FLT190-02

FLT190-02 was a multicenter, long-term, follow-up study of participants with Fabry disease who had undergone dosing with FLT190 in earlier clinical studies. Participants dosed as part of a FLT190 treatment protocol were invited to participate in this long-term, follow-up study.

Participants were asked to provide consent for this long-term, follow-up study prior to completion of the preceding study (including participants who withdrew from the preceding study for any reason). Providing consent was obtained, participants rolled over to the long-term, follow-up study upon conclusion of the preceding study.

Data on safety, durability of αGLA response, general health status, and health resource utilization were planned to be collected up to Month 60 (Year 5) post-treatment with comparisons made to the participants ' baseline status prior to dosing with FLT190.

All safety data (AEs and Serious AEs) were collected for the duration of the study.

Concomitant medications were not collected, unless they were Fabry treatments (i.e., ERT or PCT) and/or immunosuppressive treatments for vector-associated transaminitis which were collected throughout the study.

3.2 Randomization

These are non-randomized, open-label studies. All enrolled participants were to receive a single dose of FLT190 during FLT190-01.

3.3 Hypothesis Testing

There was no formal statistical hypothesis testing for the dose finding FLT190-01 study, nor for the long-term follow-up FLT190-02 study.

3.4 Interim Analysis

An interim analysis was planned in the FLT190-01 protocol when dose escalation is complete in PTP's in Part 1 and all patients have at least 12 weeks of data following FLT190 administration. The aim of this interim analysis was to inform the recommended dose for a Phase 3 study in PTPs.

However, for the purpose of an abbreviated clinical report, no interim analyses were conducted.

3.5 Sample Size Calculation/Determination of Sample Size

A total of up to 15 participants were planned to be enrolled in protocol FLT190-01. However, only 3 participants were enrolled prior to early termination of the study by the sponsor. The sample size for this study was based on clinical considerations and not formal hypotheses testing. There was no target sample size for protocol FLT190-02 since it was a long-term follow-up study for FLT190-01 patients.

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3.6 Administration of Study Medication

FLT190 was administered as a single dose in Protocol FLT190-01, via a slow intravenous infusion over 1-2 hours.

The planned dose escalation scheme was as follows (if participant weight is >90 kg, the dose will be calculated based on 90 kg weight):

- Cohort 1: 0.75 x 10^{12} vector genomes [vg] per kilogram (kg) of body weight
- Cohort 2: 1.5 x 10^{12} vg/kg
- Cohort 3: 4.5 x 10^{12} vg/kg
- Cohort 4: 1.3 x 10^{13} vg/kg

Based on the observed safety and αGLA expression within a dosing cohort, an intermediary dose may be selected for the next cohort (i.e., up to, but not above, the proposed dose of the next cohort) and the dose within a cohort may be reduced.

No dosing was planned for Protocol FLT190-02.

3.7 Blinding

These studies were open-label and as such blinding was not applicable.

3.8 Study Procedures and Flowchart

The schedule of assessments for the studies are available in the study protocols.

4. ANALYSIS POPULATIONS

4.1 Screened Set

The Screened Set includes all the participants who signed informed consent in the FLT190-01 study and all the participants in the FLT190-02 study. This will be the primary population for all the analyses.

5. GENERAL ASPECTS FOR STATISTICAL ANALYSIS

5.1 General Methods

This section describes broad statistical considerations and analysis definitions that apply to all or some of the analysis sections that follow. In general:

- All analyses will be conducted using SAS® (SAS Institute Inc., Cary, NC, USA) statistical software version 9.4 or later.
- All data collected for the FLT190-01 and FLT190-02 protocols will be provided in listings, except data collected only for study administrative purposes. If any treated participant is found to not have valid documented informed consent, that participant's data will be still included in the report, except as necessary to document the error.
- Listings will be presented by dose level and sorted by participant number and assessment date (and time) if applicable.

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- Unless otherwise specified, the data collected in both FLT190-01 and FLT190-02 will be listed combined with the study ID, to indicate from which study the data was collected.

5.2 Key Definitions

5.2.1 Study Day Definition

The study day is defined relative to the date of dose of study product (FLT190), calculated as follows:

- If the date of assessment/event is prior to the first infusion date of study drug, then

Study Day = date of assessment/event – first infusion date of study drug

- If the date of assessment/event is on or after the first infusion date of study drug, then

Study Day = date of assessment/event – first infusion date of study drug + 1

This study defines Day -1 as the day before the first dose of study product and Day 1 as the day of study product administration. There is no Day 0.

All data will be displayed in listings or figures reflecting the collected visit on CRF pages. No analysis visit windows will be defined in this SAP.

5.2.2 First and Last Dose Date

As FLT190-01 is a single dose study, the first and last dose date is defined as Day 1, when FLT190 is infused.

5.2.3 Baseline and End of Study

Unless otherwise specified, baseline is defined as the last non-missing observation prior to study treatment administration in study FLT190-01. Baseline is expected to be at Study Day -1 or Screening if the Day -1 data are not available. Both FLT190-01 and FLT190-02 will take the same baseline.

The End of Study time point will be defined as the last visit by the participant through FLT190-01 and FLT190-02 combined, which is expected to be Year 5, or sooner in the case of premature discontinuation.

5.3 Missing Data

In general, all missing data will be maintained as missing, and analyses will be performed considering all data observed.

Regarding the laboratory data with values below the quantification limit (BQL), no imputation will be done, and the values will be displayed as collected.

All other missing data will be maintained as missing unless specified otherwise.

5.4 Missing Dates

For AEs and concomitant medications, no imputation will be done for partial/missing start and end dates.

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5.5 Repeated or Unscheduled Assessments

All assessments from scheduled and unscheduled visits will be included in respective listings.

5.6 Subgroup Analysis

Due to the small sample size, subgroup analysis will not be performed.

6. PROTOCOL DEVIATIONS

All data available for protocol deviations, including deviation category, action taken, and deviation types will be listed for the Screened Set.

7. DISPOSITION, DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

7.1 Inform Consent, Eligibility and Participant Disposition

All participant level disposition data in the FLT190-01 and FLT190-02 studies, including informed consent and re-consent dates, end of study status, last study date, last study day, and follow-up duration in days will be listed for the Screened Set.

Eligibility criteria violations and screening failures (including date and primary reason for failure) will be listed for the Screened Set.

7.2 Demographics and Baseline Characteristics

All demographic and baseline characteristic data will be listed for the Screened Set. These data include age (years), height (cm), weight (kg) and body mass index (BMI, in kg/m^2) at screening in FLT190-01 and FLT190-02. Race, ethnicity, highest educational status, career status and patient employment collected at the screening of FLT190-01 will be listed.

BMI will be calculated as follows and will be rounded to 1 decimal place:

$$\text{BMI } (kg/m^2) = \text{Weight } (kg) \;/\; [\text{Height } (m)]^2$$

7.3 Fabry Disease History

All Fabry disease history data collected at the screening of FLT190-01, including age at diagnosis of Fabry disease, αGLA Genotype/Mutation, clinical signs and symptoms, and start date of Fabry disease will be listed for the Screened Set.

7.4 Prior and Concomitant Medications

Prior and concomitant medications will be collected throughout the study only for the FLT190-01 protocol. Prior and concomitant medications will not be collected for FLT190-02 protocol, unless they are Fabry treatments (i.e., ERT or PCT) and/or immunosuppressive treatments for vector-associated transaminitis, which will be collected throughout the study.

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Prior and concomitant medications will be coded by the Anatomical Therapeutic Chemical (ATC) classification system according to the World Health Organization Drug Dictionary (WHODD), version Sep 2018.

Prior medications will be defined as medications documented on the CRF as having stopped prior to the time of FLT190 infusion.

Concomitant medications will be defined as medications documented on the CRF as ongoing or having started on or after the time of FLT190 infusion.

All prior and concomitant medications will be listed for the Screened Set.

7.5 Fabry Disease Treatments

Prior and current ERT and PCT will be collected at the screening and throughout the study of FLT190-01 and FLT190-02. ERT and PCT will not be coded using WHODD ATC classification system. All data available will be listed for the Screened Set, including ERT or PCT, therapy name, therapy dose, therapy frequency, therapy start and stop date time, ongoing or not, associated infusion reactions and details.

ERT and PCT data will also be listed with plasma αGLA level for the Screened Set.

7.6 Corticosteroids and Tacrolimus Treatment

Following treatment with FLT190, liver function tests will be monitored intensively, and all participants will be provided with a take-home pack of corticosteroids and tacrolimus, which will only be used at the direction of the investigator. In addition, from the Week 3 visit, all participants will take a tapering course of corticosteroids and tacrolimus. If at any other timepoint a participant develops an increase of alanine aminotransferase (ALT) followed by a second repeat increased ALT, they will be instructed by the investigator to initiate treatment.

Corticosteroids and immunosuppressives will not be coded using WHODD ATC classification system.

All data collected on the corticosteroid and immunosuppressive related CRF page will be listed for the Screened Set, including distribution and administration, medication name, start and stop date, total daily dose (mg) and route.

Any other corticosteroid and immunosuppressive recorded on prior and concomitant medications CRF page will not be listed in this section, but in the prior and concomitant medications listing.

Corticosteroid and immunosuppressive will also be listed with tacrolimus level and ALT level for the Screened Set.

7.7 Extent of Exposure

Dosing is only planned for the FLT190-01 protocol and all exposure data will be recorded on the vector infusion CRF page. All dosing data will be listed for the Screened Set, including whether administered, reason not administered, volume (mL) administered, dose level (vg/kg) assigned, doing date, start and end dosing time, whether infusion interrupted and details.

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8. EFFICACY ENDPOINTS AND ANALYSES

All efficacy figures and listings will consider all the data from both FLT190-01 and FLT190-02 protocols for the Screened Set, unless specified otherwise.

8.1 Gb3 and LysoGb3 in Plasma and Urine

Gb3 and lysoGb3 in plasma and urine will be listed, including change from baseline.

9. EXPLORATORY EFFICACY ENDPOINTS

9.1 Leukocyte and Plasma αGLA Levels

Plasma αGLA activity and concentration will be listed with change from baseline calculated where applicable. Plasma αGLA activity will also be plotted over time at the participant level.

Leukocyte αGLA activity level will be listed, including change from baseline. Leukocyte αGLA activity level will also be plotted over time at the participant level.

9.2 eGFR

All available eGFR, with change from baseline, will be listed.

9.3 Brain MRI

All data from brain MRIs will be listed. The screening measurement will be used as the baseline measurement.

Brain MRI parameters will be listed as per cardiac MRI parameters described in Section 9.4.

The brain MRI parameters that will be listed are contained in Table 9-1.

Table 9-1 Brain MRI Parameters

Parameter	Units
Lesion Volume*	mm^3
Total Number of Lesions	Numeric
Sum of Volume	cm^3
Total Number of Chronic	Numeric
Sum of cerebral white matter (CWM) Volume	cm^3

* Summaries are at the lesion level and therefore summaries will consider the total number of lesions (rather than being participant level).

Categorical brain MRI parameters will be listed at each visit, consisting of the following parameters:

- Lesion Location (Free text)
- Lesion Type (Ischemic / Hemorrhagic)
- Lesion Status (Recent / Chronic)

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9.4 Cardiac MRI

All data from cardiac MRIs will be listed. The screening measurement will be used as the baseline measurement.

For the cardiac MRI parameters, listings will be displayed by category for cardiovascular test, consisting of the following:

- Blood Pool (BP) Assessment
- T1 Relaxation Time of Contrast Agent (CA T1)
- Extracellular Volume (ECV)
- Global left ventricle-right ventricle (LV-RV) Assessment
- Global Strain
- Late Enhancement Assessment
- Regional Native T1
- Regional T2

The cardiac MRI parameters that will be displayed are contained in Table 9-2.

Table 9-2: Cardiac MRI Parameters

Category	Parameter	Laterality	Location	Units
BP Assessment	CA T1 BP			ms
BP Assessment	Native T1 BP			ms
CA T1	Apical Anterior		Heart, Ventricle	ms
CA T1	Apical Inferior		Heart, Ventricle	ms
CA T1	Apical Lateral		Heart, Ventricle	ms
CA T1	Apical Septal		Heart, Ventricle	ms
CA T1	Basal Anterior		Heart, Ventricle	ms
CA T1	Basal Anterolateral		Heart, Ventricle	ms
CA T1	Basal Anteroseptal		Heart, Ventricle	ms
CA T1	Basal Inferior		Heart, Ventricle	ms
CA T1	Basal Inferolateral		Heart, Ventricle	ms
CA T1	Basal Inferoseptal		Heart, Ventricle	ms
CA T1	Global CA T1		Heart, Ventricle	ms
CA T1	Mid Anterior		Heart, Ventricle	ms
CA T1	Mid Anterolateral		Heart, Ventricle	ms
CA T1	Mid Anteroseptal		Heart, Ventricle	ms
CA T1	Mid Inferior		Heart, Ventricle	ms
CA T1	Mid Inferolateral		Heart, Ventricle	ms
CA T1	Mid Inferoseptal		Heart, Ventricle	ms
CA T1	ROI1 Mid-Septal			
ECV	Apical Anterior		Heart, Ventricle	%
ECV	Apical Inferior		Heart, Ventricle	%
ECV	Apical Lateral		Heart, Ventricle	%
ECV	Apical Septal		Heart, Ventricle	%
ECV	Basal Anterior		Heart, Ventricle	%
ECV	Basal Anterolateral		Heart, Ventricle	%

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ECV	Basal Anteroseptal		Heart, Ventricle	%
ECV	Basal Inferior		Heart, Ventricle	%
ECV	Basal Inferolateral		Heart, Ventricle	%
ECV	Basal Inferoseptal		Heart, Ventricle	%
ECV	Global ECV		Heart, Ventricle	%
ECV	Mid Anterior		Heart, Ventricle	%
ECV	Mid Anterolateral		Heart, Ventricle	%
ECV	Mid Anteroseptal		Heart, Ventricle	%
ECV	Mid Inferior		Heart, Ventricle	%
ECV	Mid Inferolateral		Heart, Ventricle	%
ECV	Mid Inferoseptal		Heart, Ventricle	%
ECV	ROI1 Mid-Septal			
Global LV-RV Assessment	Assess of the Larg cr-sec Thick of Tiss	Bilateral		mm
Global LV-RV Assessment	Heart Chamber Volume (HCV), End Atrial Diastole			
Global LV-RV Assessment	HCV, End Atrial Systole			
Global LV-RV Assessment	HCV, End Ventricular Diastole	Left	Heart, Ventricle	ml
Global LV-RV Assessment	HCV, End Ventricular Diastole	Right	Heart, Ventricle	ml
Global LV-RV Assessment	HCV, End Ventricular Systole	Left	Heart, Ventricle	ml
Global LV-RV Assessment	HCV, End Ventricular Systole	Right	Heart, Ventricle	ml
Global LV-RV Assessment	HCV, EVD index	Left	Heart, Ventricle	ml/m²
Global LV-RV Assessment	HCV, EVD index	Right	Heart, Ventricle	ml/m²
Global LV-RV Assessment	HCV, EVS index	Left	Heart, Ventricle	ml/m²
Global LV-RV Assessment	HCV, EVS index	Right	Heart, Ventricle	ml/m²
Global LV-RV Assessment	Left Ventricular Ejection Fraction	Left	Heart, Ventricle	%
Global LV-RV Assessment	Left Ventricular Mass Index	Left	Heart, Ventricle	g/m²
Global LV-RV Assessment	Left Ventricular Mass, Estimated	Left	Heart, Ventricle	g
Global Strain	Global Circumferential Strain			
Global Strain	Global Circumferential Strain			%
Global Strain	Global Longitudinal Strain			
Global Strain	Global Longitudinal Strain			%
Global Strain	Global Radial Strain			
Global Strain	Global Radial Strain			%

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Late Enhancement Assessment	Total Mass			g
Late Enhancement Assessment	Total Volume			ml
Late Enhancement Assessment	Volume			%
Regional Native T1	Apical Anterior		Heart, Ventricle	ms
Regional Native T1	Apical Inferior		Heart, Ventricle	ms
Regional Native T1	Apical Lateral		Heart, Ventricle	ms
Regional Native T1	Apical Septal		Heart, Ventricle	ms
Regional Native T1	Basal Anterior		Heart, Ventricle	ms
Regional Native T1	Basal Anterolateral		Heart, Ventricle	ms
Regional Native T1	Basal Anteroseptal		Heart, Ventricle	ms
Regional Native T1	Basal Inferior		Heart, Ventricle	ms
Regional Native T1	Basal Inferolateral		Heart, Ventricle	ms
Regional Native T1	Basal Inferoseptal		Heart, Ventricle	ms
Regional Native T1	Global Native T1		Heart, Ventricle	ms
Regional Native T1	Mid Anterior		Heart, Ventricle	ms
Regional Native T1	Mid Anterolateral		Heart, Ventricle	ms
Regional Native T1	Mid Anteroseptal		Heart, Ventricle	ms
Regional Native T1	Mid Inferior		Heart, Ventricle	ms
Regional Native T1	Mid Inferolateral		Heart, Ventricle	ms
Regional Native T1	Mid Inferoseptal		Heart, Ventricle	ms
Regional Native T1	ROI1 Mid-Septal			
Regional T2	Apical Anterior		Heart, Ventricle	ms
Regional T2	Apical Inferior		Heart, Ventricle	ms
Regional T2	Apical Lateral		Heart, Ventricle	ms
Regional T2	Apical Septal		Heart, Ventricle	ms
Regional T2	Basal Anterior		Heart, Ventricle	ms
Regional T2	Basal Anterolateral		Heart, Ventricle	ms
Regional T2	Basal Anteroseptal		Heart, Ventricle	ms
Regional T2	Basal Inferior		Heart, Ventricle	ms
Regional T2	Basal Inferolateral		Heart, Ventricle	ms
Regional T2	Basal Inferoseptal		Heart, Ventricle	ms
Regional T2	Mid Anterior		Heart, Ventricle	ms
Regional T2	Mid Anterolateral		Heart, Ventricle	ms
Regional T2	Mid Anteroseptal		Heart, Ventricle	ms
Regional T2	Mid Inferior		Heart, Ventricle	ms
Regional T2	Mid Inferolateral		Heart, Ventricle	ms
Regional T2	Mid Inferoseptal		Heart, Ventricle	ms
Regional T2	ROI1 Basal Inferolateral			

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10. SAFETY AND TOLERABILITY

10.1 Adverse Events

The time period for reporting AEs will be from the time the initial informed consent form (ICF) is signed by a participant until completion of the study for both FLT190-01 and FLT190-02 protocols. This includes events occurring during the screening phase of the study, regardless of whether FLT190 is administered. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding system (version 23.0) and listed by SOC and PT. AEs will be graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 or assessed as mild, moderate or severe.

Treatment emergent adverse events (TEAEs) are defined as any AEs that begin or worsen (higher grading if events are CTCAE graded, or a more severe intensity) on or after the administration of FLT190. In the event that it is not possible to determine the worsening of an event (e.g., a participant has the same event pre-infusion and post-infusion which is CTCAE graded prior to infusion and graded as mild/moderate/severe following infusion) the event will be considered treatment emergent. Events will be considered treatment emergent unless there is clear evidence to suggest that they are not. Designation of events as treatment emergent will be based upon investigator's discretion collected on CRF. Missing data will be displayed as missing.

All AEs will be included in a by-participant listing.

AE duration (days) will be included in the listings, defined as AE end date – AE start date + 1.

All information on all AEs (treatment emergent or otherwise) will be listed. No separate listings will be displayed for deaths, serious TEAEs and treatment emergent events of special interest.

10.2 Laboratory Evaluations

Laboratory tests will be performed at a central laboratory and local laboratory to assess safety.

The following laboratory tests will be performed at a central laboratory to assess safety:

- **Liver Function Tests:** albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein.
- **Hematology:** complete blood count with differential (hemoglobin, hematocrit, red blood count, white blood count, neutrophils, lymphocyte, monocytes, eosinophils, basophils), platelet count. INR, APTT, PT, platelets and hemoglobin will be measured at the screening visit (prior to renal/skin biopsies). The analysis may be conducted by the local or central laboratory.
- **Chemistry:** sodium, potassium, chloride, phosphate, CO_2, glucose, blood urea nitrogen, serum creatinine, cystatin C, C-reactive protein, troponin-T and NT-proBNP.

The following laboratory tests will be performed at a local laboratory to assess safety (local laboratory analysis will be utilized to ensure rapid turnaround of results):

- **Liver Function Tests:** albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, ALT, and AST.
- **Hematology:** complete blood count with differential (hemoglobin, hematocrit, red blood count, white blood count, neutrophils, lymphocyte, monocytes, eosinophils, basophils).

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- **Chemistry**: sodium, potassium, phosphate, blood urea nitrogen, serum creatinine, C-reactive protein, and troponin (I or T) depending on local assay availability.

All laboratory data, including results in the original units, range status, clinical significance of results, results in SI units and change from baseline, will be listed.

10.3 Vital Signs

The following vital signs parameters will be collected:
- Systolic Blood Pressure (SBP) (mmHg)
- Diastolic Blood Pressure (DBP) (mmHg)
- Pulse Rate (beats/min)
- Respiratory Rate (breaths/min)
- Temperature (°C)

Height will be collected at screening and weight will be collected at screening and on Day -1.

All vital sign data will be listed.

10.4 12-lead ECG

The overall results of the 12-lead ECGs will be recorded as normal, abnormal not clinically significant (NCS) or abnormal clinically significant (CS), at each visit.

The parameters considered will be as follows:
- Ventricular heart rate (bpm)
- PR interval (msec)
- QRS interval (msec)
- QT interval (msec)
- QTcB interval (msec)
- QTcF interval (msec)

All 12-lead ECG data will be listed.

10.5 24-hour Holter Monitor

All data related to 24-hour Holter monitor will be listed.

10.6 Liver Ultrasound

The result of the liver ultrasound will be recorded as normal, abnormal NCS or abnormal CS, at each visit. All data related to liver ultrasound will be listed.

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10.7 Physical Examination

A complete physical examination identifying any abnormalities will be conducted.

The following sites will be examined: head, neck, ears, nose, throat, eyes, chest, lungs, heart, abdomen, skin, and lymph nodes. The following systems will be assessed: musculoskeletal and neurological. Other sites/systems may be examined and specified by the examiner in the CRF.

Physical examination data will be listed.

10.8 Echocardiogram

The echocardiogram interpretation will be recorded as Normal, Abnormal NCS or Abnormal CS, at each visit. All echocardiogram data will be listed.

11. OTHER ENDPOINTS

11.1 Immune Response to αGLA

Immune response to the human αGLA transgene product will be assessed by measurement of total and neutralizing antibodies up to Week 38 following systemic administration of FLT190.

Immune response to the αGLA transgene product will be assessed by measurement of the level of total and neutralizing antibodies directed against αGLA.

The presence of αGLA antibodies will be listed as a categorical variable (positive or negative). All data on immune response to the αGLA transgene product will be listed for the Screened Set.

11.2 Immune Response to AAVS3

Immune response to the AAVS3 capsid will be assessed by measurement of:

- Anti-AAVS3 antibodies.
- T-cell responses to AAVS3 capsid in peripheral blood mononuclear cells.

11.3 Clearance of Vector Genomes in Blood, Urine, Saliva, Stool, and Semen.

PCR of vector genomes (blood [plasma], saliva, urine, stool and semen) will be collected.

Clearance of vector genomes in blood (plasma), saliva, urine, stool, and semen will be listed as a categorical measure (positive or negative for AAV DNA) at each study visit. For positive samples, the numeric result will be listed.

All data on PCR of vector genomes will be listed for the screen set.

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12. CHANGES FROM METHODS PLANNED IN THE PROTOCOL

Due to the abbreviated clinical report, only a limited number of listings and figures will be created in this SAP. Therefore, analyses specified by the protocols (FLT190-01 and FLT190-02) are not included in this SAP.

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13. DATA AND ANALYTICAL QUALITY ASSURANCE

The overall quality assurance procedures for the statistical programming and analyses are described in Everest's Standard Operating Procedures. Detailed statistical and programming quality control (QC) and quality assurance (QA) procedures are documented in the Programming QC/QA Plan.

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APPENDIX 1 LIST OF TABLES TO BE PROGRAMMED FOR ABBREVIATED CLINICAL REPORT

Note: No tables will be produced for the Abbreviated Clinical Report.

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APPENDIX 2 LIST OF LISTINGS TO BE PROGRAMMED FOR ABBREVIATED CLINICAL REPORT

Disposition, Demographics, and Other Baseline Characteristics
Listing 16.2.1.1 Informed Consent and Re-consent (Screened Set)
Listing 16.2.1.2 Participant Disposition (Screened Set)
Listing 16.2.2 Protocol Deviations (Screened Set)
Listing 16.2.3.1 Demographic and Baseline Characteristics (Screened Set)
Listing 16.2.3.2 Fabry Disease History (Screened Set)
Dosing Data
Listing 16.2.4.1 Prior and Concomitant Medications (Screened Set)
Listing 16.2.4.2 Fabry Disease Treatments ECT and PCT (Screened Set)
Listing 16.2.4.3.1 Corticosteroid and Tacrolimus Distribution and Administration (Screened Set)
Listing 16.2.4.3.2 Corticosteroid and Tacrolimus with Elevated ALT and Tacrolimus Level (Screened Set)
Listing 16.2.5 FLT190 Infusion (Screened Set)
Efficacy Data
Listing 16.2.6.1 Gb3 and LysoGb3 (Screened Set)
Exploratory Endpoint Data
Listing 16.2.6.2 αGLA Activity and Concentration (Screened Set)
Listing 16.2.6.3 eGFR (Screened Set)
Listing 16.2.6.4 Cardiac MRI (Screened Set)
Listing 16.2.6.5 Brain MRI (Screened Set)
Safety Data
Listing 16.2.7 Adverse Events (Screened Set)
Listing 16.2.8.1 Liver Function Tests (Screened Set)
Listing 16.2.8.2 Hematology (Screened Set)
Listing 16.2.8.3 Chemistry (Screened Set)
Listing 16.2.9.1 Vital Signs (Screened Set)
Listing 16.2.9.2 12-lead ECG (Screened Set)
Listing 16.2.9.3 24-hour Holter Monitor (Screened Set)
Listing 16.2.9.4 Liver Ultrasound (Screened Set)
Listing 16.2.9.5 Physical Examination (Screened Set)
Listing 16.2.9.6 Echocardiogram (Screened Set)
Other Data
Listing 16.2.10.1 αGLA Immune Response (Screened Set)
Listing 16.2.10.2 AAVS3 Immune Response (Screened Set)
Listing 16.2.10.3 PCR of Vector Genome Clearance (Screened Set)

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APPENDIX 3 LIST OF FIGURES TO BE PROGRAMMED

Efficacy Data
Figure 14.2.1 Participant-Level Line Plot of Plasma αGLA (nmol/hr/mL) by Visit and Change from Baseline (Screened Set)
Figure 14.2.2 Participant-Level Line Plot of Leukocyte αGLA (nmol/hr/mL) by Visit and Change from Baseline (Screened Set)
Figure 14.2.3 Participant-Level Line Plot of Plasma LysoGb3 (nmol/L) by Visit and Change from Baseline (Screened Set)

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