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: Protocol, Version 8.1, 09 November 2021

FREELINE

Clinical Study Protocol

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
Short Title:	A Fabry Disease Gene Therapy Study (MARVEL1)
Protocol Number:	FLT190-01
Protocol Version:	Version 8.1 (09 November 2021)
Previous Version:	Version 7.1 (01 October 2020)
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Advanced Therapy Investigational Medicinal Product:	FLT190 (Concentrate for Solution for Infusion)
Indication:	Fabry disease
Phase:	1/2
IND Number:	024628

CONFIDENTIALITY STATEMENT

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1 Protocol Approval Signature Page

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

Protocol Number: FLT190-01

This study will be conducted in compliance with the clinical study protocol, adhere to the principles of the International Council for Harmonisation (ICH) guidelines for current Good Clinical Practice (GCP), and applicable regulatory requirements.

Sponsor:

[redacted] 11/10/2021

Print Name [redacted] Signature [redacted] Date

2 Investigator's Signature Page

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 (MARVEL1)

Protocol Number: FLT190-01

I have read the protocol.

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representatives.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a patient to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines (GCP), and to conduct the study in accordance with applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

Investigator:

Print Name	Signature	Date

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4 Emergency Contact Information

In the event of a serious adverse event (SAE), the investigator must complete the study SAE and AESI Reporting Form and ensure its transmission within 24 hours to the pharmacovigilance contract research organization (PV CRO). Applicable fax numbers can be found on the Safety Fax/Email Cover Sheet (sent under separate cover).

For emergency protocol- or safety-related issues, the investigator must contact the CRO Medical Monitor:

Telephone	Direct:	+48 12 646 25 03
	Mobile:	+48 606 470 249

Or if unavailable, contact the central out-of-hours group: Telephone +1 877 462 0134.

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5 List of Abbreviations

AAV	Adeno-associated virus
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under curve
CMV	Cytomegalovirus
CRO	Contract research organization
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
DLT	Dose-limiting toxicity
DMC	Data monitoring committee
DNA	Deoxyribonucleic acid
eCRF	Electronic case report form
ECG	Electrocardiogram
EOS	End of study
ERT	Enzyme replacement therapy
EU	European Union
FACIT-Fatigue	Functional Assessment of Chronic Illness Therapy Fatigue Scale
FASTEX	FAbry STabilization indEX
FIX	Factor IX
Gb3	Globotriaosylceramide
GCP	Good Clinical Practice
eGFR	Estimated glomerular filtration rate
GI	Gastrointestinal
αGLA	α-galactosidase A
GSRS	Gastrointestinal Symptom Rating Scale
HBcAb	Hepatitis B core antibody
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HCAb	Hepatitis C antibody
HCD	Host cell DNA
HIV	Human immunodeficiency virus
IB	Investigator’s Brochure
ICH	International Council for Harmonisation
ICF	Informed consent form
Ig	Immunoglobulin
IMP	Investigational medicinal product
INR	International normalized ratio
IV	Intravenous(ly)

KDIGO	Kidney Disease: Improving Global Outcomes
LFT(s)	Liver function test(s)
LysoGb3	Globotriaosylsphingosine
MRI	Magnetic resonance imaging
MSSI	Mainz Severity Score Index
NCI	National Cancer Institute
NHP	Non-human primate
NIMP	Non-investigational medicinal product
PBMC	Peripheral blood mononuclear cells
PCP	Pneumocystis pneumonia
PCR	Polymerase chain reaction
PCT	Pharmacological chaperone therapy
PUPs	Previously untreated patients
PTPs	Previously treated patients
PTT	Partial thromboplastin time
PV	Pharmacovigilance
QoL	Quality of life
RASB	Renin angiotensin system blocker
rcAAV	Replication-competent AAV
SAR	Serious adverse reaction
SAE	Serious adverse event
SFBPI	Short Form Brief Pain Inventory
ss	Single stranded
SUSAR	Suspected, unexpected, serious adverse reaction
TB	Tuberculosis
UPCR	Urine protein to creatinine ratio
vg	Vector genomes
VZV	Varicella zoster virus

6 Protocol Synopsis

Protocol Number: FLT190-01	**Drug:** FLT190 (Concentrate for Solution for Infusion)

Study 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

Number of Subjects:

Part 1: Up to 12 previously treated patients (PTPs) will be enrolled in the study across up to 4 dose cohorts.

Part 2: 3 previously untreated patients (PUPs) will be enrolled at the dose selected in Part 1 of the study.

Sites and Regions: This global multicenter study will include approximately 18 study sites. This is a country-specific protocol for the conduct of the study in the USA.

Clinical Phase: 1/2

Rationale:

Fabry disease is a rare, X-linked inborn error of glycosphingolipid metabolism caused by an abnormal gene encoding the α-galactosidase A (αGLA) enzyme. The αGLA enzyme is ubiquitously expressed throughout the body and is responsible for the breakdown of glycosphingolipids, deficiency of which results in the accumulation of specific glycosphingolipids that are associated with the pathophysiology of the disease. Those most studied are the major substrate of α-galactosidase A, globotriaosylceramide (Gb3), and its deacetylated form, globotriaosylsphingosine (lysoGb3).

'Classic' Fabry disease manifests as multiple symptoms followed by progressive dysfunction of multiple vital organ systems and shows wide phenotypic variability. Children with classic Fabry disease present with pain, sweating abnormalities, gastrointestinal (GI) problems, and skin lesions. In adults, disease progression causes damage to the kidneys, cardiac, and cerebrovascular systems. Fabry disease is associated with major disability, organ failure and premature death, generally before the age of 60 years. Later onset variants present later in life with organ dysfunction which can be as severe as in classical forms of the disease, but without the peripheral stigmata of pain, sweating abnormalities and angiokeratoma.

Current treatment for Fabry disease is limited to the symptomatic management of pain, conventional management of complications, and methods to increase the availability of functional αGLA. Currently there are two available approaches for increasing the availability of functional αGLA, enzyme replacement therapy (ERT), and a more recently approved pharmacological chaperone therapy (PCT), the latter limited to patients with an 'amenable' mutation (approximately 35-50% of patients with Fabry disease). The goal of both forms of treatment is to limit the intracellular accumulation of Gb3 and lysoGb3 thereby preventing further organ damage. ERT involves infusions, every 2 weeks, of recombinant or gene engineered αGLA, while the chaperone therapy is oral.

ERT is typically administered initially in the hospital setting and then, in approximately 50% of cases, subsequently administered at the patient's home by a homecare nurse, the patient, or a relative. The short half-life of administered αGLA enzyme in the circulation and the 2-weekly infusion cycle leads to a saw-tooth pharmacokinetic profile with high levels immediately following infusion which fall away to very low levels within days. This profile may limit the ability of ERT to achieve maximal therapeutic benefit.

FLT190 is an advanced therapy investigational medicinal product intended for the treatment of Fabry disease. It is a recombinant adeno-associated viral (AAV) vector (AAVS3) containing the human αGLA gene as a single stranded (ss) deoxyribonucleic acid (DNA). Continuous endogenous synthesis of αGLA by the hepatocytes after a single administration of gene therapy is expected to lead to constant high levels of αGLA, rather than the saw-tooth pattern of conventional ERT, releasing patients from the burden of frequent infusions. Because of the constant high levels, the αGLA exposure will be much

greater than that achieved with conventional ERT. This offers the potential for improved clearance of Gb3/lysoGb3, greater therapeutic effect, and better patient outcomes than current treatments.

This study aims to investigate the safety of a new gene therapy (FLT190) and its potential to reduce and prevent cellular accumulation of Gb3/lysoGb3 in patients through the endogenous production of αGLA following a single administration of vector.

Inclusion and Exclusion Criteria:

Inclusion Criteria:

1. Adult males, ≥18 years of age with classic Fabry disease.
2. Confirmed diagnosis of classic Fabry disease (including historical documentation of a classic pathological GLA mutation).
3. Plasma αGLA activity at screening (measured by central laboratory activity assay at trough) less than 5% of normal according to the central laboratory reference range.
4. One or more of the characteristic features of classic Fabry disease: neuropathic pain, corneal verticillata, clustered skin angiokeratoma.
5. Estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73m^2 at screening, per serum creatinine, using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.
6. <500 mg/g urine protein to creatinine ratio (UPCR) in a spot urine sample OR if ≥500 mg/g, <1 g/24 hours of urinary protein (24-hour urine analysis), at screening.
7. Provision of full informed consent and able to comply with all requirements of the study, and willingness to consider participating in the 5-year long-term follow-up.
8. Willingness to practice barrier contraception as specified in the protocol.
9. Lack of neutralizing anti-AAVS3 antibodies using an in vitro transduction inhibition assay within 6 weeks prior to vector administration.
10. For inclusion in Part 1, subjects must have received either a licensed ERT or PCT for at least 12 months prior to FLT190 dosing, at a stable dose (subjects receiving both ERT and PCT will be ineligible). For inclusion in Part 2, subjects must have never previously received treatment with either ERT or PCT (licensed or investigational).
11. Willingness to avoid strenuous exercise during first 3 months after FLT190 dosing.

Exclusion Criteria:

1. Presence of GLA mutations leading to non-classical Fabry disease manifestation and any mutations that have not yet been classified[a].
2. Prior hypersensitivity or intolerance to ERT.
3. Prior lack of response to ERT.
4. Subjects with a history of chronic kidney disease stages 3-5 (Kidney Disease: Improving Global Outcomes [KDIGO] 2012 classification), documented in medical records for a minimum of 3 months.
5. Subjects with myocardial fibrosis (≥3 segments) identified by magnetic resonance imaging (MRI) at screening.
6. Use of investigational therapy for Fabry disease within 60 days before enrolment. In addition, participation in any other clinical study of an investigational medicinal product (IMP), and/or receiving any other IMP during the study.
7. Persistently elevated alanine aminotransaminase (ALT) or aspartate aminotransferase (AST); or bilirubin >1.5 x upper limit of normal, during screening.

8. Platelet count <100 x 10^9/L.
9. Subjects receiving warfarin or other anticoagulants interfering with the ability to perform renal or skin biopsies, or subjects with a clinically significant bleeding disorder.
10. A history of Hepatitis B infection, or a positive serology test at screening for hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb), or negative for hepatitis B surface antibody (HBsAb).
11. A positive serology test at screening for hepatitis C antibody (HCAb) with a positive HCV-RNA load assay, or currently undergoing anti-viral therapy for hepatitis C.
12. A negative test at screening for anti-varicella zoster virus (VZV) IgG.
13. Either history of HIV infection, or a positive serology test at screening for human immunodeficiency virus (HIV).
14. A history of tuberculosis, or a positive screening test for tuberculosis. The screening test may be repeated once following an indeterminate result.
15. Subjects who have received live attenuated vaccination within 12 weeks prior to screening or intend to receive such vaccination during the study.
16. Uncontrolled glaucoma, diabetes mellitus, or hypertension.
17. History of any malignancy requiring treatment.
18. History or detection of significant arrhythmia on screening assessments with ECG/24-hour Holter monitoring. A significant arrhythmia includes those deemed clinically significant by the investigator e.g. advanced heart block (2^{nd} or 3^{rd} degree), supraventricular or ventricular arrhythmias.
19. Subjects with uncontrolled cardiac failure, unstable angina, myocardial infarction or other cardiac presentations deemed significant by the investigator in the past 6 months.
20. History of acute myocarditis or presence of acute myocarditis during screening.
21. Prior treatment with any gene therapy medicinal product.
22. Known or suspected intolerance, hypersensitivity, or contraindication to gadolinium, tacrolimus and other macrolides, steroids, local anesthetics used for skin or renal biopsies, the IMP, and non-investigational medicinal products (NIMPs) or their excipients.
23. Subjects who are assessed as having any contraindications to MRI. Including subjects with ferromagnetic metallic implants, including pacing and defibrillator devices, nerve stimulators and cochlear implants.
24. Subjects who have had a renal transplant.
25. Cytomegalovirus (CMV) immunoglobulin (Ig)G positive subjects who are CMV polymerase chain reaction (PCR) positive at screening.
26. Current or relevant history of a physical or psychiatric illness or any medical condition that in the opinion of the investigator could affect the subject's safety or interfere with the study assessments.
27. History of substance abuse including alcohol abuse or alcohol dependence.

[a] Refer to Fabry disease mutation database to determine classic and non-classical mutations. E.g. International Fabry Disease Genotype-Phenotype Database. If there is any uncertainty around the mutation classification discuss with Medical Monitor.

Methodology:

This is a Phase 1/2, open-label, baseline-controlled, non-randomized, single-ascending dose study of FLT190 in patients with Fabry disease. Subjects who provide consent to participate in this study will be screened for eligibility up to an 18-week period.

On the day prior to infusion (Day -1), the subject will attend the investigational site and final eligibility assessments will be conducted. On Day 0, FLT190 will be administered as a single-dose, slow intravenous infusion into a peripheral vein, and the subject will be monitored closely for at least 8 hours following the infusion. Following satisfactory results from safety evaluations conducted on Day 0, the subject will be discharged from the investigational site but will continue to be monitored through a comprehensive set of safety assessments at outpatient visits for a period of 38 weeks; following which, the subject will be invited to enter a period of long-term follow-up conducted under a separate protocol.

The study will be conducted in 2 parts:

Part 1: Up to 4 dose cohorts of vector will be tested in a dose escalation scheme in previously treated patients (PTPs). PTPs are 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. Subjects receiving both ERT and PCT will be ineligible to participate in the study. In Part 1, eligible patients currently receiving ERT will maintain their current treatment until a minimum of 2 consecutive results at least 4 weeks post-dosing show αGLA expression higher than pre-dose trough αGLA expression, after which ERT may be withdrawn. In Part 1, eligible patients currently receiving PCT will have 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 will be observed. Dose cohorts are planned to include between two and three PTPs depending on the occurrence of adverse events (AEs) and/or αGLA expression observed following treatment. Safety outcomes (as determined by AE reporting and laboratory tests) and results of αGLA expression, will be evaluated at several time points. An interim analysis is planned when dose escalation is complete in PTPs in Part 1 and all patients have at least 12 weeks of data following FLT190 administration. The aim of this interim analysis is to inform the recommended dose for a Phase 3 study in PTPs. When the Part 1 dose escalation is complete (and all patients have at least 12 weeks of data following FLT190 administration), dosing in Part 2 will also be discussed with the DMC to inform the recommended dose for Part 2 in PUPs. Subjects in the USA will only be enrolled for Cohort 2 and above.

Part 2: A single cohort of three PUPs will be enrolled at the dose level selected in Part 1. PUPs are defined as patients who have never previously received treatment with ERT or PCT (licensed or investigational).

On completion of the study, all subjects will be invited to enter a separate long-term follow-up study to be followed-up for a total of 5 years after dosing.

Cohort Expansion and Dose Escalation Rules (Part 1)

Subjects will be treated in a staggered approach.

Safety and αGLA assessments for dose escalation and cohort expansion rules will take place at least 8 weeks post-dosing for subjects in Cohort 1 and 2.

Safety and αGLA assessments will take place at least 8 weeks post-dosing for the first (sentinel) subject in Cohort 3 and 4.

Safety and αGLA assessments will take place at least 4 weeks post-dosing for the second and subsequent subjects in Cohort 3 and 4.

A cohort may be expanded to include additional subjects, to further inform the safety and efficacy of any given dose level. Dosing in those additional subjects will be staggered by at least 4 weeks post FLT190 administration.

An interim analysis is planned when dose escalation is complete in PTPs in Part 1 and all patients have at least 12 weeks of data following FLT190 administration. The aim of this interim analysis is to inform the recommended dose for a Phase 3 study in PTPs. When the Part 1 dose escalation is complete (and all patients have at least 12 weeks of data following FLT190 administration), dosing in Part 2 will also be discussed with the DMC to inform the recommended dose for Part 2 in PUPs.

Subjects in the USA will only be enrolled for Cohort 2 and above.

Dose escalation will be overseen by a Data Monitoring Committee (DMC) in accordance with the following rules:

In Cohorts 1 and 2, at least two subjects will be dosed in each cohort. Depending on assessment of safety, tolerability and αGLA expression, additional subjects may be treated.

In Cohorts 3 and 4, three subjects will be dosed in each cohort.

The sponsor may expand a dose cohort (including a dose reduction) after discussion with the DMC to better characterize the safety profile or αGLA expression.

Upon dose escalation, the sponsor may increase to an intermediary dose (higher than current cohort but lower than the dose level of the next planned cohort) after discussion with the DMC.

1. Safety Assessment

Assess safety first to check for tolerability and AEs. The following rules will be applied to safety:

After first subject has been dosed in a cohort:

- If no dose-limiting toxicity (DLT) occurs, follow dosing rules in relation to αGLA expression and dose a second subject in the cohort.
- If a DLT occurs in the first subject: follow dosing rules in relation to αGLA expression and dose a total of three subjects in the cohort.

After two subjects have been dosed in a cohort:

- If no DLT occurs in either subject at the dose level, follow dosing rules in relation to αGLA expression and inter-subject variability.
- If a DLT occurs in either subject, follow dosing rules in relation to αGLA expression and dose a third subject.
- If a DLT occurs in both subjects: consult with the DMC to reduce dose before dosing a third subject.

If a third subject has been dosed:

- If no DLT occurs, follow dosing rules in relation to αGLA expression.
- If a DLT occurs in the third subject, with another DLT observed in either the first or second subject, consult with DMC to reduce dose, before dosing the next subject as part of the next cohort.
- If DLT occurs in third subject, but no DLTs occurred in first or second subject, follow dosing rules in relation to αGLA expression.

In Part 2, safety assessment will be based on the total number of subjects that experience a DLT at a specific dose level across Parts 1 and 2, in accordance with the rules above.

Note: A DLT is defined as any severe AE at least possibly related to FLT190 except for increases in ALT or AST that are not associated with increases in bilirubin. Transaminase increases should be investigated to exclude other causes but it is expected that such transaminase increases will be cases of vector associated transaminitis which is an event anticipated with liver directed AAV gene therapy. In the case of vector associated transaminitis, where ALT or AST levels are classed as severe then changes to the prophylactic immunosuppressive regimen will be considered for ongoing subjects and before treating further subjects.

2. αGLA Expression (Plasma αGLA Activity/Concentration)

- If the safety assessment does not result in a change to the planned dose escalation or cohort expansion, αGLA expression will be assessed, through plasma αGLA activity and/or concentration

to mitigate the risk of dosing additional subjects at potentially sub-therapeutic or beyond known tolerated αGLA expression.

- Subtherapeutic αGLA expression:
  - Plasma αGLA expression that is within the normal range is currently considered insufficient to drive optimal cellular uptake. Estimated normal range of αGLA activity is 4 to 22 nmol/h/mL (dependent on laboratory assay).
  - On review of data following dosing with FLT190:
    - In Cohorts 1 and 2, if plasma αGLA expression is less than the ULN in subjects, provided safety assessment permits, the study may advance to the next cohort.
    - In Cohorts 3 and 4, three subjects will be dosed in each cohort.
- Beyond known tolerated αGLA expression:
  - αGLA expression >2400 nmol/h/mL indicates beyond known tolerated αGLA expression.
  - On review of data following dosing with FLT190, if a subject reaches αGLA expression >2400 nmol/h/mL, no further subjects will be dosed at that or a higher dose. The DMC will be consulted to reduce dose before dosing the next subject.

3. <u>αGLA Expression (Inter-subject Variability)</u>
   - In Cohorts 1 and 2, if αGLA expression in the second subject is similar to the first subject in the cohort (αGLA expression with plasma αGLA activity within ± 20% of each other), then the study will advance to the next dose cohort provided the safety and αGLA expression requirements in rules 1 and 2 above are satisfactorily addressed.
   - If αGLA expression is not similar but satisfies the requirements of the cohort expansion rules (1 and 2 above), then a third subject will be recruited to the cohort. After this, the study will advance to the next dose if the safety assessment and the αGLA level dose escalation rules are met.

Objectives:

Primary:

- To investigate the safety of systemic administration of FLT190 in adult males with Fabry disease.

Secondary:

- To investigate endogenous production of αGLA following systemic administration of FLT190 in adult males with Fabry disease.
- To investigate the clearance of Gb3 and lysoGb3 measured in plasma and urine.
- To establish a baseline for long-term follow up of cellular Gb3 inclusions in skin and renal biopsies.
- To assess viral shedding in various body fluids after systemic administration of FLT190.
- To describe the immune responses to the endogenous product of αGLA following systemic administration of FLT190.

Exploratory:

- 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.
- To investigate the impact of endogenous production of αGLA on leukocyte αGLA levels.
- To investigate the impact of endogenous production of αGLA on eGFR.

- To investigate the impact of endogenous production of αGLA on urinary protein and albumin excretion.
- To investigate the impact of endogenous production of αGLA on cardiac parameters.
- To investigate the impact of endogenous production of αGLA on ischemic/hemorrhagic lesions and regions of chronic white matter hyperintensities within the brain.
- To investigate the impact of endogenous production of αGLA on quality of life.
- To investigate the impact of endogenous production of αGLA on health resource utilization.
- To investigate the impact of endogenous production of αGLA on symptomatic pain.
- To investigate the impact of endogenous production of αGLA on 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.
- To describe the immune response to AAVS3 capsid proteins following the systemic administration of FLT190.

Investigational Product, Dose Cohorts, and Mode of Administration:

FLT190 will be given as a single dose, slow intravenous infusion over 1-2 hours.

The planned dose escalation scheme is as follows (if subject 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

Subjects in the USA will only be enrolled for Cohort 2 and above.

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.

Non-Investigational Medicinal Products:

Following treatment with FLT190, liver function tests will be monitored intensively, and all subjects 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 subjects will take a tapering course of corticosteroids and tacrolimus. If at any other timepoint a subject develops an increase of ALT followed by a second repeat increased ALT they will be instructed by the investigator to initiate treatment.

Primary Safety Endpoint:

- Safety as assessed by the reporting of AEs.

Secondary Endpoints:

Safety

- Safety as assessed by reporting of abnormal or change from baseline findings from safety assessments including, laboratory assessments, vital signs, ECG, 24-hour Holter monitoring, echocardiogram, cardiac MRI, physical examination, and liver ultrasound.

Efficacy

- Change from baseline in Gb3 and 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 antibodies up to Week 38 following systemic administration of FLT190.

Shedding

- Clearance of vg in blood, urine, saliva, stool, and semen.

Pharmacokinetic

- Change from baseline in plasma αGLA activity and concentration at Week 12, 24, and 38.
- Overall αGLA activity and concentration area under curve from baseline to Week 38.

Exploratory Endpoints:

Efficacy

- Change from baseline in leukocyte αGLA 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 ERT or 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-Fatigue scale (FACIT-Fatigue).
- Change from baseline in blood plasma immune biomarkers.

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.

Health-related Quality of Life

- Change from baseline in quality of life score using the EQ-5D-5L questionnaire at Week 38.

Health Resource Utilization

- Change from baseline in the level of health resource utilized by the subjects, 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 subjects and caregivers (e.g., for ERT dosing).

Maximum Duration of Subject Involvement in the Study:

- Planned duration of screening period: up to 18 weeks
- Planned duration of follow up period post infusion: 38 weeks
- Planned duration of long-term follow-up: a total of 5 years from dosing (under a separate long-term follow-up protocol).

Table 1: Schedule of Assessments

Procedure	Week	Day[B]					Week[B]																	
	Screening[A]	-1[P]	0	+1	+2	+5	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	28	32	38/ EOS
Visit Window	-18 to -1 weeks	-2 day				± 1 day	± 1 day												± 3 days						
Informed consent	X	Days -1 to +5 see Table 2																							
Demography & medical history	X																								
Fabry disease, ERT & PCT history	X																								
Prior & concomitant medications	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE assessment	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination[C]	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[D]	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
24-hour Holter monitor	X									X						X									X
Echocardiogram	X									X				X				X		X					
Liver ultrasound	X																								X
SFBPI – diary completion[E]	X									X								X				X			X
FACIT-Fatigue & GSRS – diary completion[F]	X									X								X				X			X
Cardiac & brain MRI	X																								X
MSSI & FASTEX[G]	X																								X
Skin/renal biopsy[H]	X																								
QoL (EQ-5D-5L)	X																								X
Health resource utilization	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prophylactic immunosuppressants[I]									→											→					
Local Laboratory Tests																									
LFTs (local)							X[J]	X[J]	X[J]	X[J]	X[J]	X[J]	X[J]	X[J]	X[J]	X[K]	X[K]	X[K]	X[K]	X[K]	X[K]	X[J,K]	X[J]	X[J]	X
Troponin (I or T per available local assays)	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Test for CMV[L]									→											→					
Tacrolimus level (trough)[M]									→											→					

Procedure	Week	Day[B]					Week[B]																	
	Screening[A]	-1[P]	0	+1	+2	+5	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	28	32	38/ EOS
Visit Window	**-18 to -1 weeks**	**-2 day**				**± 1 day**	**± 1 day**												**± 3 days**						
Central Laboratory Tests																									
Anti-AAVS3 neutralizing antibody test[N]	X	Days -1 to +5 see Table 2																							
Hematology, chemistry incl. LFTs, hs troponin-T & NT-proBNP	X[O]						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis[P]	X																								
Serology[Q]	X																								
TB screen	X																								
αGLA plasma activity & concentration level	X[R](trough)						X J	X J	X J	X J	X J	X J	X J	X J	X J	X K	X K	X K	X K	X K	X K	X J,K	X J	X J,S	X
αGLA leukocyte activity level	X[R](trough)									X								X				X			X
αGLA immune response	X						X	X	X			X						X				X			X
Gb3/lysoGb3 plasma & urine (Part 1)	X							X		X		X		X				X		X	X	X	X	X	X
Gb3/lysoGb3 plasma & urine (Part 2)	X						X	X	X	X		X		X				X		X	X	X	X	X	X
AAVS3 immune response (antibodies)							X	X				X						X				X			X
AAVS3 immune response (T-cell responses)							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mononuclear cells (research)										X								X							X
eGFR[T]	X									X				X				X		X	X	X	X	X	X
Biomarker & research plasma samples							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Research serum samples							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Research urine samples																									X
Urine protein & albumin[U]	X									X				X				X		X	X	X	X	X	X
24-hour urine analysis[V]	X																								
PCR of vector genomes: plasma, saliva, urine, stool, & semen[W]							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: AAVS3=adeno-associated virus; AE=adverse event; CMV=cytomegalovirus; ECG=electrocardiogram; EOS=end of study; ERT=enzyme replacement therapy; FACIT-Fatigue=Functional Assessment of Chronic Illness Therapy Fatigue Scale; FASTEX=FAbry STabilization indEX; Gb3=globotriaosylceramide; eGFR=estimated glomerular filtration rate; $_{\alpha}$GLA=α-galactosidase A; GSRS=Gastrointestinal Symptom Rating Scale; hepB=hepatitis B; hepC=hepatitis C; HIV=human immunodeficiency virus; hs troponin-T=high-sensitivity troponin-T; Ig=immunoglobulin; incl=including; INR=International normalized ratio; LFTs=liver function tests; lysoGb3=globotriaosylsphingosine; MRI=magnetic resonance imaging; MSSI=Mainz Severity Score Index; NT-proBNP=N-terminal prohormone of brain natruiretic peptide; PCR=polymerase chain reaction; PCT=pharmacological chaperone therapy; PTPs=previously treated patients; PTT=partial thromboplastin time; QoL=quality of life; SFBPI=Short Form Brief Pain Inventory; TB=tuberculosis, VZV= varicella zoster virus.

[A] For subject screening, multiple visits may be required to perform and repeat assessments and prepare for enrolment.

[B] At the investigator's discretion, Week 1 through Week 32 visits may be conducted at home with a visiting nurse performing assessments and sample collection. Visits Days -1, 0, +1, +2, and +5 to be conducted at the infusion study site.

[C] The following sites will be examined: head, neck, ears, nose, throat, eyes, chest, lungs, heart, abdomen, skin, and lymph nodes. Weight will also be measured. Height, waist circumference (cm), hip circumference (cm), neck circumference (cm), and bioimpedance will be measured at screening.

[D] Vital signs include pulse, blood pressure, respiratory rate, and temperature.

[E] Subjects will complete SFBPI questionnaire once a day during the screening period, and then for 7 consecutive days, on each occasion commencing at the start of Weeks 4, 12, 24, and 37 (week prior to the 38-week visit). Study sites will contact subjects at the beginning of Week 37 to remind them to complete the questionnaires during Week 37.

[F] Subjects will complete the FACIT-Fatigue and GSRS questionnaires at the end of each week during the screening period, and then at the end of Weeks 4, 12, 24, and 37.

[G] FASTEX assessment will be completed following the completion of the cardiac MRI analysis.

[H] Skin and renal biopsy to be conducted when all eligibility-determining screening assessments have been completed and subject has been confirmed as eligible to participate in the study.

[I] A weight-based tapering immunosuppressive regimen will be initiated at the Week 3 visit (Section 11.4).

[J] Testing to be performed twice weekly (including the testing as part of the scheduled study visit, initially commencing at Week 1 and concluding at the end of Week 9, and later commencing at Week 27 and concluding at the end of Week 37. The tests should be as evenly spaced through the week as possible, for example: Monday and Thursday, or Tuesday and Friday.

[K] Testing to be performed three times weekly (including the testing as part of the scheduled study visit) commencing at Week 10 and concluding at the end of Week 26. The tests should be as evenly spaced through the week as possible, for example Monday, Wednesday, and Friday.

[L] Local testing. For patients positive for CMV (IgG) at screening, weekly CMV PCR testing for the duration of the immunosuppressant regimen. Tests should also be undertaken to coincide with use of immunosuppression if outside of the prophylactic regimen. On days where other clinical assessments are not required these samples may be taken at study site or alternative location (e.g. patient's home). Management guidelines in the case of CMV reactivation can be found in Appendix 4.

[M] Local testing. Tacrolimus trough levels should be tested twice weekly until levels are in the desired range and weekly thereafter for the duration the patient is taking tacrolimus in the immunosuppressant regimen. Tests should be undertaken to coincide with use of tacrolimus if outside of the prophylactic regimen. On days where other clinic assessments are not required these samples may be taken at study site or at an alternative location (e.g. patient's home). Management guidelines for tacrolimus dosing can be found in Appendix 5.

[N] A negative assay outcome must be documented within 6 weeks of dosing. It is acceptable to repeat samples during the screening period as necessary. Two aliquots will be taken at the time point.

[O] INR/PTT, and hemoglobin will also be measured during the screening period (prior to the renal/skin biopsies) as part of the pre-biopsy safety assessment (analyzed by either the local or central laboratory).

[P] Urinalysis will be performed during the screening period (prior to the renal biopsy) to confirm absence of urine infection.

[Q] Serology will be conducted by a central laboratory and will screen for hep B (surface antigen, core antibody, and surface antibody), hepC (antibody and PCR [hepC PCR only indicated for a positive HCAb screen]), HIV 1 and 2 antibodies, and VZV IgG. CMV IgG testing followed by CMV PCR (CMV PCR only indicated for a positive CMV IgG).

[R] In Part 1 (PTPs) the αGLA blood sample is to be collected within 48 hours prior to the patient's next scheduled ERT dosing.

[S] αGLA plasma activity testing (central) to continue up until the completion of Week 37.

[T] eGFR will be derived using the eGFR creatinine-cystatin C formula by the central laboratory as part of the chemistry safety assessment.

[U] Subjects will collect separate urine samples (first morning, void/mid-stream) on the day of the site visit and the 2 days preceding the site visit.

[V] 24-hour urine collection only required if screening urine spot analysis indicates protein/creatinine ratio is >500 mg/g.

[W] Three times within 7-10 days following vector infusion then weekly until the results from all matrices are negative at three consecutive visits. Semen samples may be omitted at the discretion of the PI for religious or other personal reasons expressed by the subject.

Table 2: Detailed Schedule of Assessment for Infusion Week

Procedure	Day	Day 0 (Infusion Day)										Day		
		Pre-dose[A]	Timepoint During Infusion		Timepoint (hours) from End of Infusion									
	-1	-1h	0	Every 15 min	+1	+2	+3	+4	+5	+6	+8[B]	+1	+2	+5
Window	-2 days			± 5 min	± 10 min						± 15 min			± 1 day
Physical examination[C]	X											X	X	
Vital signs[D]	X	X		Every 15 min	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X											X	X	X
FLT190 administration			X	→										
Prior & concomitant medications	X	X	X	Every 15 min	X	X	X	X	X	X	X	X	X	X
AE assessment	X	X	X	Every 15 min	X	X	X	X	X	X	X	X	X	X
SFBPI, GSRS, FACIT-Fatigue – diary completion	X													
Local Laboratory Tests														
SARS-CoV-2 (COVID-19)[E]	X													
Hematology, chemistry[F]	X	X								X		X	X	
LFTs[G]	X	X								X		X	X	X
Central Laboratory Tests														
Hematology, chemistry incl. LFTs & hs troponin-T	X[H]												X	
αGLA plasma activity and concentration levels		X												X
αGLA leukocyte activity level		X												
AAVS3 immune response (antibodies)	X													
AAVS3 immune response (T-cell responses)	X													
αGLA immune response	X													
Anti-AAVS3 neutralizing antibody test	X													
Mononuclear cells (research)	X													
Biomarker & research plasma sample	X													
Research serum sample	X													
eGFR[I]	X													
Gb3/lysoGb3 plasma & urine	X													
Urine protein & albumin[J]	X													
Research urine sample	X													
PCR of vector genomes: plasma, saliva, urine, stool, & semen	X												X	X

Abbreviations: AAVS3=adeno-associated virus; AE=adverse events; CRP=C-reactive protein; ECG=electrocardiogram; eGFR=estimated glomerular filtration rate; FACIT-Fatigue=Functional Assessment of Chronic Illness Therapy Fatigue Scale; Gb3=globotriaosylceramide; $_{\alpha}$GLA=α-galactosidase A; GSRS=Gastrointestinal Symptom Rating Scale; hs troponin-T=high-sensitivity troponin-T; LFTs=liver function tests; lysoGb3=globotriaosylsphingosine; min=minutes; PCR=polymerase chain reaction; SFBPI=Short Form Brief Pain Inventory.

[A] Approximately 1 hour before infusion.

[B] Following 8-hour post-dosing monitoring, additional 2 hourly monitoring of vital signs may occur if deemed necessary by the investigator.

[C] The following sites will be examined: head, neck, ears, nose, throat, eyes, chest, lungs, heart, abdomen, skin, and lymph nodes. Weight, waist circumference (cm), hip circumference (cm), neck circumference (cm), and bioimpedance will be measured at Day -1.

[D] Vital signs from Day -1 to Day +2 include pulse, blood pressure, respiration rate, and temperature.

[E] SARS-CoV-2 (COVID-19) testing should be conducted by local laboratories in line with current local practices within 72 hours prior to FLT190 dosing (Section 13.1.3).

[F] Complete blood count with differentials, sodium, potassium, phosphate, blood urea nitrogen, serum creatinine, CRP.

[G] Albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase, aspartate aminotransferase.

[H] Amylase and hs troponin-T levels will be measured at Day -1 as part of the central laboratory analysis.

[I] eGFR will be derived using the eGFR creatinine-cystatin C formula by the central safety laboratory using the cystatin C and creatinine results analyzed as part of the chemistry safety assessment.

[J] Subjects will collect separate urine samples (first morning, void/mid-stream) on the day of the site visit and on the 2 days preceding the site visit.

7 Introduction

7.1 Background

Fabry disease belongs to a group of at least 50 genetically distinct, biochemically-related lysosomal storage disorders [Germain 2010]. Each disorder is cause by an inborn error of metabolism due to a monogenetic defect resulting in the deficiency of lysosomal enzyme(s) [Germain 2010]. Fabry disease is an X-linked lysosomal storage disorder caused by a mutation in the gene encoding the α-galactosidase A (αGLA) enzyme [Ortiz 2018]. A marked reduction or an absence of GLA activity results in progressive accumulation of glycolipids in plasma and in a wide range of cells throughout the body [Ortiz 2018]. This progressive accumulation triggers a cascade of cellular dysfunction [Germain 2010].

Fabry disease affects mainly males, but also heterozygote females with the defective gene, where a high percentage of females develop vital organ involvement about a decade later than males [Germain 2010]. Median age for the onset of symptoms is between 3 and 10 years for males and between 6 and 15 years for females [Mehta 2010]. Organ complications tend to emerge in young adults [Ortiz 2018]. Fabry disease is associated with reduced life expectancy of 50 years for men and 70 years for women [Mehta 2010, Germain 2010].

Reported incidences of Fabry disease ranging from 1 in 476,000 to 1 in 117,000 in the general population [Germain 2010]; however, new-born screening initiatives have found a higher prevalence of the disease (1 in 3,100 new-borns in Italy) [Germain 2010].

Fabry disease is a multi-system condition and symptoms express themselves in many organs. Therapy for Fabry disease includes enzyme replacement therapy (ERT), conventional medical, and adjunctive therapies, which consist of supportive therapies [Germain 2010]. Current guidelines recommend ERT to reduce symptoms and prevent late-stage disease complications [Ortiz 2018]. Pharmacological chaperone therapy (PCT) is approved in the USA, Europe, and Canada for the treatment of a subset of Fabry patients with amenable mutations; however, clinicians have not yet gained sufficient clinical experience to make recommendations on use of this treatment [Ortiz 2018].

7.2 FLT190

FLT190 is an advanced therapy investigational medicinal product (ATIMP) intended for the treatment of Fabry disease. It is a recombinant adeno-associated viral (AAV) vector (AAVS3) containing the human *GLA* gene as a single stranded (ss) deoxyribonucleic acid (DNA). It has three major features (Figure 1).

1. A small synthetic human liver specific promoter (FRE1)
2. The transgene (GLA)
3. A novel engineered capsid (AAVS3) which has been selected based on its ability to preferentially transduce human hepatocytes with high efficiency and specificity



Figure 1: Schematic Representation of the DNA Construct

The construct package within and delivered to the target cells in an AAVS3 capsid (ITR: Inverted terminal repeat-2; FRE1 liver specific promoter; BgpA bovine growth hormone polyadenylation signal sequence; GLAco codon-optimized α-galactosidase A SV40i: SV40 intron)

FLT190 is administered by peripheral vein infusion and will act by using a viral vector AAVS3 to transfer a functional copy of the *GLA* gene to host cells. Uptake into hepatocytes is receptor-mediated [Mingozzi 2011]. With AAV gene therapy the transgene largely remains episomally in the transduced cells. Under the control of the liver specific FRE1 promoter the *GLA* gene will be expressed in host hepatocytes leading to the production of functional αGLA enzyme. The functional αGLA enzyme produced will be released from hepatocytes to the circulation from where it will be taken up from the plasma by host cells in non-hepatic tissues where it will lead to hydrolysis of terminal α-galactosyl moieties from glycosphingolipids. Clearance of glycosphingolipids using ERT has been shown to lead to amelioration of symptoms [Germain 2010]. Therefore, it is expected that the αGLA produced by FLT190 will lead to treatment of the recipient's symptoms.

7.3 Non-Clinical Data

The overall non-clinical development program consists of in vitro and in vivo pharmacodynamic studies and a single-dose Good Laboratory Practice-compliant toxicology/biodistribution study in rhesus macaques. Non-clinical studies showed the FRE1 promoter is highly liver specific and that the AAVS3 capsid is efficient at transducing human hepatocytes. In Fabry knock out mice, αGLA levels in plasma reached a high steady state resulting in normalization of globotriaosylceramide (Gb3) and globotriaosylsphingosine (lysoGb3) and a reduction in lipid deposits in kidney and cardiac tissues. Stable endogenous αGLA levels were measured at 1- and 3-month follow-up. In non-human primates (NHP), high levels of αGLA were observed, but very low/no neutralizing antibodies, demonstrating that liver expressed αGLA due to the restricted promoter/enhancer (FRE1) was associated with reduced host immune responses. A single administration of FLT190 was well tolerated in rhesus monkeys and there were no adverse findings up to 13-weeks post dose.

Refer to the FLT190 Investigator's Brochure (IB) for full details of the non-clinical data.

7.4 Clinical Data

The study represents the first administration of FLT190 to humans.

Refer to the FLT190 IB for full details of the clinical data.

7.5 Rationale and Risks/Benefits

7.5.1 Rationale

Current treatment for Fabry disease is limited to the symptomatic management of pain, conventional management of complications, and methods to increase the availability of functional αGLA. Currently there are two available approaches for increasing the availability of functional αGLA, ERT, and a more recently approved PCT, the latter limited to patients with an 'amenable' gene mutation (approximately 35-50% of patients with Fabry disease) [Hughes 2017]. The goal of both forms of treatment is to limit the intracellular accumulation of Gb3 and lysoGb3 thereby preventing further organ damage. Enzyme replacement therapy consists of recombinant or gene engineered αGLA to decrease levels of Gb3/lysoGb3 and prevent further disease progression and is typically administered initially in a hospital setting and then in, approximately 50% of cases, subsequently administered at the patient's home by a homecare nurse, the patient or a relative. The short half-life of ERT in the circulation and the 2-weekly infusion cycle leads to a saw-tooth pharmacokinetic profile with high levels immediately following infusion which fall away to very low levels within days. Pharmacological chaperone therapy is administered orally.

Fabry disease is a compelling disorder for gene therapy because of the continuing unmet need in altering the disease course, despite the availability of ERT and PCT, which can stabilize progression of the Fabry disease. Gene therapy with FLT190 is expected to deliver higher and consistent levels of αGLA, as seen in non-clinical studies. A single administration of FLT190 has the potential to improve the clearance of Gb3 from tissues and thereby significantly improve patient outcomes.

FLT190 offers the following potential clinically relevant advantages for Fabry disease patients:

- A single treatment with FLT190 is expected to lead to long-term production of supraphysiological levels of functional αGLA enzyme, avoiding the need of chronic administration of medication.
- Based on the fact, that αGLA will be generated in the subject's own hepatocytes, the enzyme will have the subject's individualized glycosylation pattern. As such the potential for antibody development including the potential for the development of neutralizing antibodies is reduced compared to ERT [Mingozzi 2003]. This is supported by data from NHP studies (refer to IB for more information).
- Liver expression as a result of AAV gene transfer induces immune tolerance to secreted liver expressed human proteins. Tolerance induction is favored by higher levels of transgene expression [Mingozzi 2003].
- The continuous production of functional αGLA enzyme will avoid the brief peaks and prolonged troughs in plasma αGLA levels that are seen with ERT and is expected to lead to improved clearance of glycosphingolipids and thereby an improvement in efficacy.

This first-in-human clinical study aims to investigate the safety of FLT190 and the potential to alter the disease phenotype through the endogenous production of αGLA following a single administration of the vector.

Dose Selection

The objective is to select an initial dose of FLT190, based on FLT190 studies in NHP and extrapolation from results from Freeline's experience with FLT180a, which shares the same promoter and capsid as FLT190 that will result in αGLA activity levels that would, at a minimum, reduce disease severity whilst balancing the risk of toxicity.

Literature evidence indicates that higher αGLA levels and, more specifically, greater cumulative dose or exposure, leads to reduced Gb3 and lysoGb3, reduced symptoms, greater clinical efficacy and a reduction in podocyte Gb3 deposits in patients [Skrunes 2017; Tondel 2012; Smid 2011; Aasebø 2010]. In vitro αGLA uptake experiments demonstrate that high exogenous αGLA is required to reach maximal intracellular αGLA concentrations [Shen 2016; Lee 2003; Johnson 2016] as summarized in the FLT190 IB. Therefore, steady state αGLA plasma levels significantly greater than physiological levels (Section 7.5.2) and exposure exceeding that of current approved ERT (Table 3) is required to drive cellular uptake into the target cells and reduce the lysosomal storage of Gb3 in target organs such as the kidney.

Table 3: Exposure to αGLA

Product	**C_{max} ng/mL**	**†C_{max} nmol/h/mL**	**$AUC_{0-\infty}$ mg.min/mL**	**AUC mg.min/mL 1 year**
Fabrazyme 1 mg/kg	2100-3500	4200-7000	0.4-0.8	10.4-20.8
Replagal 0.2 mg/kg	nd	nd	*0.0075	1.95

Abbreviations: AUC=area under the curve; C_{max}=maximum concentration; nd=not disclosed.

* Calculated from clearance (Cl)(male) 2.66 mL/min/kg at 0.2 mg/kg dose (European Public Assessments Report) Cl=Dose/AUC.

† Calculated based on 1:2 GLA ng/mL:GLA nmol/h/mL relationship [Tsukimura 2014].

The level of expression in animals is variable, so cautious prediction of human expression from animal studies necessitates the inclusion of a range for potential expression. With the dose response linear at lower levels of vector [Nathwani 2009] we predict the following for the low and high dosing levels.

For comparison the normal level of αGLA is approximately 7nmol/h/mL, making the exposure (area under the curve [AUC]) seen with currently approved ERTs approximately equivalent to normal levels for Replagal and 4 to 8 times normal for Fabrazyme. For FLT190 the dose level of 0.75 x 10^{12} vector genomes (vg)/kg has been selected as it is considered to be a safe starting dose and is 20-fold less than the non-clinical toxicology testing dose for which there were no FLT190 related adverse findings (see FLT190 IB for non-clinical safety data). This dose is predicted to provide 0.6-fold normal αGLA activity. The second dose of 1.5 x 10^{12} vg/kg is predicted to deliver 1.3-fold normal αGLA activity. The third dose of 4.5 x 10^{12} vg/kg is predicted to deliver 3.8-fold normal αGLA activity. The fourth dose of 1.3 x 10^{13} vg/kg is predicted to deliver 11-fold normal αGLA activity. The potential for liver expression from FRE1-containing AAVS3 products in NHP can be up to 250-fold the predicted levels for FLT190, however our expectation based on expression for FLT190 in NHP and first patient data dosed at 0.75 x 10^{12} vg/kg is as predicted above. The starting dose is anticipated to provide therapeutic benefit, whilst the next dose levels are anticipated to provide sufficient αGLA to drive cellular uptake to achieve intracellular αGLA activity levels in the normal range.

The risk of exposure to supraphysiological levels of αGLA are addressed in Section 7.5.2.

Dosing and Dose-escalation Interval

To monitor for acutely occurring, dose-limiting adverse events (AEs) and to minimize the risk of markedly exceeding known tolerated αGLA levels a flexible interval between subjects will be implemented.

Subjects will be treated in a staggered approach. Intervals between dosing subjects will be enforced to monitor for acutely occurring dose-limiting AEs. Greater than 2400 nmol/h/mL indicates beyond known tolerated αGLA expression. If αGLA expression exceeds 2400 nmol/h/mL, no further subjects will be dosed at that or a higher dose. The Data Monitoring Committee (DMC) will be consulted to reduce dose before dosing the next subject. It is expected that the levels of αGLA activity achieved with FLT190 will be well below that of the peak levels (C_{max}) of conventional ERT and of those under development.

In Part 1, eligible patients currently receiving ERT will maintain their current treatment until a minimum of 2 consecutive results at least 4 weeks post-dosing show αGLA expression higher than pre-dose trough αGLA expression, after which ERT may be withdrawn. In Part 1, eligible patients currently receiving PCT will have 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 will be observed.

The AAVS3 capsid containing a Factor IX (FIX) transgene has previously been administered in hemophilia subjects, and no acutely occurring significant adverse reactions have been observed. The potential risks and benefits of FLT190 are explained in Section 7.5.2.

As subject follow-up will potentially be carried out across multiple sites, it will be a requirement for all investigators with active subjects who have received FLT190 to confirm the absence of safety issues in subjects under their care that would prevent further subject dosing/dose escalation. Dosing of subsequent subjects will not be allowed until this confirmation is received.

Renin Angiotensin System Blocker Concomitant Treatment

As part of this study Renin Angiotensin System Blocker (RASB) concomitant treatment is required for subjects with evidence, at screening or in their medical history, of the equivalent of 500 mg/24 hours (spot urine protein to creatinine ratio (UPCR) in mg/g approximates protein excretion in mg/24h; spot UPCR 500 mg/g, approximates to 500 mg/24hr protein excretion) or more of urinary protein.

The rational for this requirement is that treatment with a RASB is becoming increasingly standard of care for subjects with proteinuria >500 mg/24hr, and if treatment was to commence during the follow up period of the study the RASB would influence urinary protein excretion and glomerular filtration rate. This makes the analysis of the relationship of these endpoints and their relationship to FLT190 treatment difficult to interpret.

7.5.2 Potential Risks and Benefits

The following are potential risks associated with participation in this study:

Humoral Immune Response to AAV

Experience in animal models indicates that administration of AAV results in a serotype -specific stimulation of neutralizing antibody production, and that such antibodies preclude re--administration of AAV of the same, or related, serotype. It may be that serotypes other than AAVS3 could be successfully re-administered after treatment with FLT190 but this depends on how related they are to AAVS3. It is highly probable that subjects who receive AAVS3 whilst participating in this study will develop neutralizing antibodies and that any future administration of vector of this, or related, serotype would be ineffective. This event becomes more problematic if the individual subject receives a sub-therapeutic dose. The Sponsor's objective is therefore to select an initial dose, based on studies in NHP and extrapolation from results in previous studies that may result in αGLA levels that would, at a minimum, reduce disease severity (Section 7.5.1). In FIX studies where a similar capsid containing a FIX transgene was administered in hemophilia B patients by Nathwani, et al, it has been demonstrated that FIX activity remains stable (for up to 8 years as of June 2018) but as the gene is maintained episomally, natural turnover of liver cells suggested that it is possible for levels to decline over time [Nathwani 2018]. An initial therapeutic level therefore might become subtherapeutic in the future, increasing the importance of careful evaluation of benefit/risk in dose selection. A similar response for αGLA protein production and levels is expected to occur following FLT190 dosing.

A major mitigating factor against the risk of patients in the FIX studies developing immunity to the capsid was that in both the murine and NHP models, vector particles of an alternative serotype could be administered with subsequent predictable expression of FIX [Ljung 1999]. Therefore, if patients were enrolled into the FIX studies and received a sub-therapeutic dose, they could be considered for future studies of AAV vectors of an alternate serotype with a potentially therapeutic benefit despite the presence of antibodies to AAVS3. It is expected that Fabry disease subjects that receive the AAVS3 vector may also be able to receive vector particles of an alternative serotype and subsequently achieve predictable expression of αGLA.

Vector-associated Transaminitis and Loss of αGLA Expression

One of the medium-term safety issues associated with AAV vectors from prior clinical studies include transaminase elevation, which can be associated with permanent loss of expression of the transgene. In five clinical studies performed with AAV vectors in hemophilia B patients, 13 experienced transient transaminase elevation in cohorts dosed from 4×10^{11} vg/kg to 2×10^{13} vg/kg [Manno 2006, Nathwani 2014, Miesbach 2017, George 2016, Monahan 2015], that may be associated with a permanent decline in FIX levels, suggesting a loss of transduced hepatocytes. Increase in alanine aminotransaminase (ALT) requiring steroid treatment has occurred as early as 4 weeks post-treatment [George 2016] but is more generally acknowledged to occur 7-12 weeks after gene transfer. Transaminitis requiring prednisolone treatment did not occur until the highest dose of 2×10^{12} vg/kg had been administered in the study [Nathwani 2014]. There have been two cases of transaminitis requiring prednisolone treatment among patients treated at 5×10^{11} vg/kg in the Phase 1/2 clinical study with SPK-9001 [George 2016]. Furthermore, transaminitis has been seen in association with AAV8, AAVrh74, AAVrh10 and AAV5.

This transaminitis appears to be more of an efficacy 'risk' than a true safety risk because the transaminase elevation and accompanying decline in therapeutic protein levels have to date been asymptomatic and self-limited. Experience has shown that the risk can be well managed through the rapid administration of immune suppression but a delay in the introduction of corticosteroids however has been linked to loss of expression in earlier clinical studies [Kumar 2016].

To mitigate against this risk of transaminitis and subsequent loss of αGLA expression in this study, subjects will be monitored intensively over the course of the study. All subjects will commence a prophylactic course of immunosuppressants commencing from Week 3. In addition, subjects will be given a take-home pack of immunosuppressants to be used in the event of transaminase elevation outside of this time, to facilitate rapid initiation of treatment if necessary. Subjects will be instructed to not take these immunosuppressants unless told to do so by the investigator.

Immunosuppressants to Prevent and Treat Transaminitis

The long-term use of high dose corticosteroids may be associated with the development of side effects. These include susceptibility to infections, high blood pressure, elevation in blood sugar, weight gain with increased appetite, reduced fertility in men, and fluid retention. Osteoporosis is generally considered a long-term side effect but may, in its most severe form, evolve to aseptic necrosis or pathological fractures.

Cramps and joint pain have also been described. The occurrence of glaucoma and cataracts has been described in patients taking long-term corticosteroids. In addition, gastrointestinal (GI) irritation may occur and could result in GI hemorrhage. Emotional disturbances and mood changes are also described.

The use of tacrolimus may be associated with the development of side effects. These include lymphoma and other malignancies, susceptibility to infections including polyoma virus and cytomegalovirus, nephrotoxicity, neurotoxicity, hyperkalemia, hypertension, hyperglycemia, insomnia, visual disturbances, headaches, hyperphosphatemia, tremor, myocardial hypertrophy, pure red cell aplasia, hemolytic uremic syndrome, posterior reversible encephalopathy syndrome. Use of live vaccines for immunization should be avoided.

Subjects should also avoid prolonged exposure to ultra-violet light and sunlight by wearing protective clothing and sunscreen with a high protection factor (minimum Sun Protection Factor 30) and avoid grapefruit juice/ grapefruit during use of tacrolimus.

The risk of these side effects to immunosuppressants will be minimized by careful monitoring, utilization of the lowest effective dose, and tapering/cessation in the prophylaxis regimen and in response to break through transaminitis as soon as the evidence of transaminitis begins to subside. The risk of side effects to immunosuppressants are expected to be limited due to the short prophylactic course for up to 14 weeks, though this may be longer if required to treat transaminitis.

Subjects with a contraindication to immunosuppressants are excluded from the study.

Myocarditis

Mild transient myocarditis with high-sensitivity (hs) troponin-T elevation has been reported after FLT190 infusion (refer to FLT190 IB for full details).

Cardiac involvement is common in Fabry disease and manifestations include arrhythmias, left ventricular hypertrophy, valvular disease, and heart failure, and is one of the leading causes of death in Fabry disease [Linhart 2007]. Troponin levels are not routinely monitored in clinical practice and are only measured in response to clinical signs and symptoms. Continuously elevated troponin has been found in Fabry disease patients with cardiac involvement without coronary artery disease [Feustel 2014]. Immune-mediated myocarditis is seen in Fabry-related cardiomyopathy. In a case series of 78 adult patients with Fabry-related cardiomyopathy, 56% had histologic evidence of myocarditis across all phases of hypertrophy, including the pre-hypertrophic phase [Frustaci 2018].

Vector Shedding and Germline Transmission

Following systemic administration of FLT190 there is potential for the spread of vector particles to non-hepatic tissues including the gonads. Therefore, shedding will be assessed in samples taken three times over the first 7-10 days of the study. Thereafter samples will be taken weekly until no further evidence of FLT190 is detectable (as determined by negative results from all matrices at three consecutive visits at least 1 week apart). As a precaution, only subjects willing to practice a reliable barrier method of contraception during the study will be enrolled to avoid the possibility of horizontal, and potentially vertical, transfer of vector.

Development of Neutralizing Anti-human αGLA Antibodies

A significant concern of this study, as with previous studies, is that subjects may develop an immune response to human αGLA protein following systemic administration of FLT190.

Theoretically antibodies could be raised to the secreted protein if the host did not have experience with it, for example if they had no endogenous protein (cross-reactive immunologic material negative) or the protein was foreign. Enzyme replacement therapy does elicit an immune response in a significant portion of Fabry patients treated with enzyme therapy [Wilcox 2012].

Liver directed gene therapy production of αGLA, however, is less likely to result in emergence of or an increase in neutralizing antibodies for the following reasons.

First, αGLA will be generated in the patient's own hepatocytes, the enzyme will have the subject's individualized glycosylation pattern. As such the potential for antibody development including the potential for the development of neutralizing antibodies is reduced compared to ERT [Mingozzi 2003].

Second, AAV liver directed gene therapy can harness the unique pro-tolerogenic properties of the liver, termed the liver tolerance effect, to induce peripheral immune tolerance to the transgene product (GLA). FLT-190 not only targets liver cells preferentially over other tissues for transduction due to the tropism of the capsid, AAVS3, but also contains a liver-specific regulatory element (FRE1) within the expression cassette which limits transgene expression to hepatocytes.

Several preclinical studies indicate that endogenous synthesis of human FIX and hFVIII [Nathwani 2001; Mingozzi 2003, Limmer 2000], αGLA [Nietupski 2011] and other antigens [Sharland 2010; Herzog 2011] in the liver induce immune tolerance and reduce the risk of developing neutralizing antibodies. Further, liver-directed AAV gene therapy has been shown to eradicate preexisting anti-cFIX antibodies and induced immune tolerance in an inhibitor-prone hemophila B canine model [Crudele 2015].

The greatest clinical experience with AAV liver directed gene therapy is in trials involving patients with hemophilia B, where more than 100 patients with hemophilia B have been given gene therapy to date across multiple studies and no inhibitors (neutralizing antibodies) have been reported [Arruda 2016]

In this study, subjects who have a history at screening of prior hypersensitivity or intolerance to ERT or prior lack of response to ERT, the clinical sequelae of anti-GLA antibodies, will be excluded from the study. Study halting criteria include the development of neutralizing αGLA antibodies associated with a decreased clinical response, such as a decrease in GLA activity and an increase in serum lysoGB3 levels.

In summary, the risk of the development or worsening of anti-GLA immune response with subsequent loss of efficacy from rescue ERT, is thought to be low, and similar in both previously treated and treatment naïve patients.

Insertional Mutagenesis and/or Tumorigenesis

Insertional mutagenesis leading to oncogenesis is a recognized safety concern of vector -based gene therapy medicinal products. For vectors that do not efficiently integrate, such as AAV vectors modified to avoid integrations, insertions into the genome represent unintended and probably rare events.

Deep sequencing studies in murine and nonhuman primate models have shown that integration of the AAV genome can occur in the liver [Nowrouzi 2012; Li 2011]. Additionally, studies in mice have reported an increased incidence of hepatocellular carcinoma following perinatal or neonatal gene transfer [Donsante 2007; Chandler 2015]. Studies in other murine models of bleeding diathesis however have failed to replicate this finding and collectively the available data in mice as well as larger animal models suggest that AAV has an extremely low risk of tumorigenesis [Kay 2007].

Overall, the risk of insertional mutagenesis following AAV-mediated gene transfer has been assessed to be low. Proviral DNA is maintained predominantly episomally in the transduced cell and the assessment of low risk is consistent with the fact that wild-type AAV infection in humans, although common, is not associated with oncogenesis. Subjects will be followed for up to 5 years under a separate long term follow up protocol and will be fully informed regarding risk of mutagenesis prior to study entry.

Exposure to Supraphysiological Levels of αGLA

The FLT190 dose of 1.3 x 10^{13} vg/kg is predicted to deliver maximally 11-fold normal exposure levels and is well within known tolerated αGLA levels based on clinical experience with existing ERTs and a long-acting ERT in development, further described below.

If an αGLA level is reached that is predicted to lead to steady state αGLA levels that exceeds overall exposure experienced with ERTs, the dosing interval will be increased to enable the subject to be observed for a longer period to assure confidence in the stabilized level, and a dose reduction may be considered for future subjects, in accordance with Section 14.3.

Literature evidence indicates that higher αGLA levels and, more specifically, greater cumulative dose or exposure, leads to reduced Gb3 and lysoGb3, reduced symptoms, greater clinical efficacy and a reduction in podocyte Gb3 deposits in patients. Therefore, steady state αGLA plasma levels significantly greater than physiological levels are required to drive cellular uptake into the target cells and reduce the lysosomal storage of Gb3 in target organs such as the kidney. Data on the safety of supraphysiological levels of αGLA is available from three sources:

1. Naturally occurring αGLA over-expression

In humans, naturally occurring sustained over-expression of αGLA has been observed at 2- to 7-fold normal levels and occurs due to a promoter mutation [Saifudeen 1995; Fitzmaurice 1997]. This high plasma activity allele was inherited as an X-linked trait affecting only this lysosomal enzyme with a frequency of 0.5% among 395 normal males and despite these higher levels of αGLA expression it resulted in a normal phenotype without reports of adverse effect.

2. Existing ERT

The safety of high levels of αGLA can also be imputed from the clinical safety profile of the existing ERTs, Fabrazyme and Replagal. The peak levels seen with these ERTs are of the order of 36 times that which is anticipated to be achieved with the proposed dose of FLT190 at 1.3 x 10^{13}vg/kg but quickly drops to very low levels, with annual exposure levels of 1 to 8-fold normal. Despite this very high peak level the risk-benefit ratio of these ERTs was considered positive and generally both are well-tolerated with the majority of AEs described as mild to moderate in nature with the most significant risks of immunogenicity, hypersensitivity, or infusion-associated reactions.

3. Long-acting ERT in development

The FLT190 dose of 1.3 x 10^{13} vg/kg is predicted to deliver maximally 11-fold normal exposure levels. There are published clinical study results from a long-acting ERT in development [Schiffman 2019] with a C_{max} around 5000-fold normal and an annual exposure of 305-fold normal levels, with reports of mainly mild to moderate treatment-emergent AEs. There was a case of severe bronchospasm occurring as part of an infusion associated reaction and required treatment with adrenaline but had a satisfactory outcome with the patient discharged from the hospital the day following the reaction. This AE occurred acutely at the time of administration and so may be linked to the high peak level achieved. The patient had pre-existing IgE antibodies to the long-acting ERT. No significant adverse effects associated with long-term treatment have been reported for this long-acting ERT.

As described above, the risk of immunogenicity may be diminished by treatment with FLT190 [Mingozzi 2003] and regular infusions will not be required with FLT190, almost completely eliminating the risk of infusion-associated reactions. On this basis, the overall risk is considered low for the plasma levels of αGLA that are anticipated to be sustained with FLT190. A conservative approach to dosing is planned, as described in Section 7.5.1.

Exposure to Suboptimal Levels of αGLA

As described in Section 7.5.1, the initial FLT190 dose level (0.75 x 10^{12} vg/kg) has been selected based on FLT190 studies in NHP and extrapolation from experience with FLT180a, which shares the same promoter and capsid as FLT190, and was initially expected to result in αGLA plasma levels that, at a minimum, reach near normal levels but potentially many fold higher. The dose escalation rules have been designed to minimize the number of subjects dosed at suboptimal levels of FLT190 by permitting early escalation if there are no safety concerns and αGLA levels are considered suboptimal.

Following FLT190 dosing, αGLA levels will be measured and subjects will be clinically monitored for signs and symptoms that may suggest suboptimal αGLA levels. For subjects previously treated with ERT (PTP) this may be similar to signs and symptoms typically experienced when trough αGLA levels are reached after intermittent ERT administration. For

previously untreated subjects (PUP), signs and symptoms are likely to be similar to those prior to receiving FLT190.

If either the predicted AUC endogenous production of αGLA is projected to fall short of levels achieved by approved ERT/PCT, or if there is clinical evidence of a lack of response, a discussion will take place between the Sponsor and the investigator to discuss any requirement to initiate ERT/PCT treatment (Sections 12.3.2 and 12.3.3).

Risk of Withdrawal of ERT/PCT

In Part 1, eligible patients currently receiving ERT will maintain their current treatment until a minimum of 2 consecutive results at least 4 weeks post-dosing show αGLA expression higher than pre-dose trough αGLA expression, after which ERT may be withdrawn. In Part 1, eligible patients currently receiving PCT will have 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 will be observed. During the study, αGLA levels will be measured and subjects will be clinically monitored for signs and symptoms that may indicate a need to restart ERT/PCT. For PTP this may be similar to signs and symptoms typically experienced when trough αGLA levels are reached after intermittent ERT administration. For PUP, signs and symptoms are likely to be similar to those prior to receiving FLT190.

If either the predicted AUC endogenous production of αGLA is projected to fall short of levels achieved by approved ERT/PCT, or if there is clinical evidence of a lack of response, a discussion will take place between the Sponsor and the investigator to discuss any requirement to initiate ERT/PCT treatment (Sections 12.3.2 and 12.3.3).

Risk of Blood Tests

The risk of blood tests from a vein includes temporary discomfort at the site of puncture, possibly bruising and swelling around the puncture site, occasionally infection and bleeding into the surrounding muscle and tissues. These complications are likely to be exceedingly rare as many Fabry disease patients are routinely administered ERT without significant procedure related side effects. Standard procedures that are well established in clinical settings in the Europe and North America will be followed to ensure the safe and uneventful administration of FLT190 and during venepuncture for blood tests.

Allergic Reactions or Anaphylaxis

Allergic-type reactions, including anaphylaxis are a rare consequence of administration of biologicals. Onset of anaphylaxis is usually rapid following injection of an allergen with 90% beginning within 40 minutes. Subjects should be informed of early symptoms and signs of hypersensitivity reactions, including hives, generalized urticaria, angioedema, chest tightness, dyspnea, wheezing, faintness, hypotension, tachycardia, and anaphylaxis. Subjects will remain under observation in the investigational center for at least 8 hours following infusion of FLT190 to minimize the risk associated with acute allergic reaction. Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored at 15-minute intervals from the start of infusion. Vital signs will be monitored hourly for 6 hours following the end of the infusion, at 8 hours, and then every 2 hours until the subject leaves the investigational center if deemed necessary by the investigator.

Subjects will be excluded at screening should they have a history of hypersensitivity or contraindication to Replagal or Fabrazyme.

Treatment management plans for anaphylaxis (and immune complex disease and infusions reactions) are provided in Appendix 1.

Risks of Renal Biopsy

The risks associated with a needle biopsy of the kidney include the possibility of significant bleeding around the kidney and pain. Such severe pain as to require narcotics for relief and/or an overnight stay happens in less than 1 out of 200 biopsies.

Blood-tinged urine occurs in one out of 10 to 15 biopsies, but this almost always clears without treatment within 24 hours. Bleeding into the urine collecting system of the kidney that causes discomfort or temporary blockage of the kidney or bladder and requires the placement of a catheter to drain the urine from the bladder may also occur in less than 1 out of 200 biopsies. Repeated episodes of bleeding due to damage to a blood vessel (artery) within the kidney may occur in less than 1 in 500 biopsies. In that case, the damaged vessel may need to be embolized through a catheter guided into the damaged artery from an artery in the leg. This would result in the permanent loss of blood flow to a small portion of one kidney but is unlikely to measurably affect overall kidney function.

The chance is less than 1 in 3,000 that surgery may needed because of bleeding or other complications following biopsy. The chance is less than 1 in 7,500 a kidney would have to be removed.

The chance of death resulting from a kidney biopsy is exceedingly rare and likely less than 1 in 10,000.

To minimize these risks, biopsy prerequisites will include normal blood pressure, hemoglobin >10 g, platelet count >150,000, normal coagulation studies, both renal lengths ³10 cm with <1.5 cm length discrepancy between them and a urinalysis showing no indications of urinary tract infection. If the subject's platelet count is >100,000 but <150,000 local procedures and medical discretion should be followed with written approval to proceed being obtained from a hematologist (or equivalent) prior to the biopsy being carried out. Only highly experienced physicians will perform kidney biopsies using sterile techniques minimizing risk of infection. If the performing physician considers that the risk of complications from kidney biopsy is higher than the usual, the physician will forgo the renal biopsy. As a minimum, the following measures should be evaluated following the biopsy: blood pressure, presence of visible blood in the urine, presence of abdominal or back pain requiring more than paracetamol/acetaminophen or a similar drug to control, finding of >1 g drop in hemoglobin 4-6 hours post biopsy and any additional standard-of-care protocols at each site. In the case of any complication the physician will inform the subject and will make decisions about further testing including computed tomography scan or blood transfusion, etc. Subjects will minimize the risks of bleeding after the biopsy by refraining from strenuous physical activity (including running) or heavy lifting for 10 days after the biopsy. If needed, a letter their employer to this effect will be provided. The risks described above are those of a biopsy being performed for the purposes of clinical care and are likely substantially less for research biopsies where many of the risk factors for complications from clinical biopsies are absent.

Risks of Skin Biopsy

The risks associated with skin biopsy are minor pain or bleeding that could be caused by the needle used for local anesthesia or the biopsy itself. Occasionally a suture (stitch) may be required to stop bleeding. In addition, there is a small risk for local skin infection that will be mitigated by proper local skin disinfection prior to the procedure. Subjects should be asked for

allergic reactions to local anesthetics as this is a preventable risk if the appropriate agent is being used. Although the biopsy taken will be small, the subject will have a small scar in the long term.

Risks of Magnetic Resonance Imaging

The test will take approximately 45 minutes. Magnetic resonance imaging (MRI) does not involve ionizing radiation and thereby has no effects on subjects or researchers.

Some subjects may feel claustrophobic when entering the MRI scanner. In such instances, subjects will be removed from the scanner immediately. The requirement to remove a subject from the scanner occurs in less than 1:20 patients.

There is a rare association between specific types of gadolinium contrast and a condition known as nephrogenic systemic fibrosis. This has only been reported in patients on dialysis, patients with acute kidney injury and when estimated glomerular filtration rate (eGFR) is below 30 mL/min/1.73m^2 (Medicines and Healthcare products Regulatory Agency Guidelines). Renal function is measured as part of the study entry requirements and such subjects are excluded from participating in the study. The gadolinium enhance contrast used in MRI imaging is used at a lower dose than in other indications and the risks of contraindications are expected to be lower. To date there have been no reported complications in the United Kingdom when these restrictions have been applied.

Risks Associated with DNA Impurities

DNA impurities in AAV vector preparations may derive from the host cells used for virus production or from the plasmids used in transient transfection. The risk related to host cell DNA (HCD) relates primarily to the possibility of undesirable (oncogenic) sequences being packaged in AAV capsids and delivered to the cell nucleus where they may integrate or form episomes. This could, in theory, allow expression of oncogenes.

Freeline have performed a detailed assessment of the risks associated with the packaging of undesirable HCD sequences and have concluded that the risk is negligible since the HCD is expected to be ss and will therefore be rapidly degraded giving little potential for recombination with the host cell genome. Given that the vast majority of a host cell genome is transcriptionally inactive, the likelihood of integration is extremely low [Reeves 1984]. The expected copy numbers of undesirable sequences (i.e. those which could be functionally expressed) which could reach the cell nuclei are expected to be very low and the risk of integration is negligible. The risk of expression of an oncogenic protein from a non-integrated HCD sequence is considered to be insignificant because these non-integrated molecules either will undergo rapid degradation or will be diluted out upon cell division.

Literature reports and Freeline data demonstrate that packaging frequencies of plasmid sequences are higher than those of HCD sequences. A potential concern relating to residual plasmid sequences (such as the AAV capsid ‘cap’ gene) being packaged in AAV particles is the possible expression of immunogenic peptides encoded by such sequences. However, Freeline data demonstrate that residual ‘cap’ sequences in highly purified AAV vector preparations are only transcribed at very low levels in in vitro transduced cells supporting the assumption that residual plasmid DNA is predominantly not expressed or expressed to a very limited extent only. The bacterial kanamycin ‘kan’ gene present in the plasmid backbone codes for resistance to the kanamycin antibiotic used during plasmid production. An 80 kg subject with Fabry disease treated at the dose of 1.3 x 10^{13} vg/kg of FLT190 containing 15% ‘kan’ sequences, i.e. at the specification limit for release, would receive approximately 1.6 x 10^{14}

copies of the kanamycin resistance gene, of which only a subset could in theory be functionally expressed. In contrast, approximately 4000-fold higher levels of functional kanamycin sequence have been delivered in naked DNA plasmid studies in humans by intramuscular administration (often repeat administration) without any observed toxicity [Alton 2015; Kastrup 2005; Buscail 2015; Romero 2004]. Moreover, this antibiotic is not routinely used in clinical practice so no adverse impact of trace amounts of functional 'kan' sequences would be anticipated. Levels of both 'cap' and 'kan' sequences are expected to be less than 15% of total genomes based on batch analyses data generated to date (and per above, only a small fraction of these could potentially be functional).

The role of bacterial DNA impurities, deriving from the plasmids, in the initiation of liver transaminitis cannot be ruled out, especially as bacterial DNA itself can generate an immune response in humans, but these could only be present in exceptionally low amounts and the transient immunosuppression with corticosteroids described in the protocol is expected to suppress liver cytotoxicity and preserve long-term transgene expression from hepatocytes.

The manufacturing process for FLT190 has been designed to reduce both host cell and plasmid DNA impurities to an acceptable level. Furthermore, quality control tests for total HCD and specific undesirable sequences (such as E1A and SV40T) as well as plasmid 'kan' and 'cap' sequences are included in the drug substance specification to ensure that these are controlled to acceptable limits.

Risk Associated with Replication Competent AAV

A concern related to replication competent AAV (rcAAV) is that cells simultaneously transduced/infected with rcAAV, recombinant AAV, and helper virus provide all the functions required to amplify the recombinant AAV vector leading to progeny formation which would increase the vector dose. However, the two-plasmid system used in the manufacture of FLT190 has been specifically designed to reduce the likelihood of rcAAV production compared with other plasmid systems and no replication has been observed in batch release testing to date (as defined by the specification criteria for no replication of <10 rcAAV in 1 x 10^8 vg). rcAAV is therefore not considered to represent a safety risk in Freeline products.

Unknown Risks Associated with First Time in Human FLT190

This is a first time in human clinical study of FLT190 and as such there may be as yet uncharacterized risks that have not been evident in preclinical species.

Since αGLA exposure is seen in healthy humans, and in Fabry disease patients receiving ERT, any FLT190 related acute toxicities are expected to be related to the AAV capsid rather than the endogenously produced αGLA. The FLT190 capsid is identical to the capsid that is currently being administered in the clinic to subjects as part of the ongoing FIX gene therapy studies [FLT180a Clintrials.gov].

A comprehensive set of safety assessments is included in the protocol but to mitigate against as yet unknown risks, the following additional steps will be taken.

All subjects will remain under observation in the investigational center for at least 8 hours post-treatment to monitor for any unknown risks. All infusion centers will have access to emergency facilities.

Subjects will be treated in a staggered approach including intervals between dosing each of the subjects, to minimize risk to subsequent subjects of unanticipated delayed AEs. The inter-subject intervals can be extended based on pre-specified rules (Section 14.3.1).

The following are potential benefits associated with participation in this study:

- A single treatment with FLT190 is expected to lead to long-term production of supraphysiological levels of functional αGLA enzyme, avoiding the need of chronic administration of medication
- Based on the fact, that αGLA will be generated in the patient's own hepatocytes, the enzyme will have the subject's individualized glycosylation pattern. As such the potential for antibody development including the potential for the development of neutralizing antibodies is reduced compared to ERT [Mingozzi 2003]. This is supported by data from NHP studies.
- Liver expression as a result of AAV gene transfer induces immune tolerance to secreted liver expressed human proteins. Tolerance induction is favored by higher levels of transgene expression [Mingozzi 2003].
- The continuous production of functional αGLA enzyme will avoid the brief peaks and prolonged troughs in plasma αGLA levels that are seen with ERT and is expected to lead to improved clearance of glycosphingolipids and thereby an improvement in efficacy.

The potential benefits are applicable to both PTPs and PUPs. Currently available treatment options include ERT (agalsidase alpha, agalsidase beta) or PCT (migalastat). Despite these treatments, there remains an unmet need due to incomplete efficacy. All products require chronic, and potentially life-long administration. Both ERT products involve intravenous (IV) infusions which can be time-consuming, particularly agalsidase beta (Fabrazyme) and may be associated with infusion reactions and immunogenicity, which may be associated with reduced efficacy. Pharmacological chaperone therapy is only available for patients with an amenable mutation, around 35-50% of patients with Fabry disease.

7.5.3 Assessment and Management of Risk

This study is categorized as: Type C = markedly higher than the risk of standard medical care.

Management plans for occurrence of certain events described above can be found in Appendix 1.

8 Objectives

8.1 Primary

- To investigate the safety of systemic administration of FLT190 in adult males with Fabry disease.

8.2 Secondary

- To investigate endogenous production of αGLA following systemic administration of FLT190 in adult males with Fabry disease.
- To investigate the clearance of Gb3 and lysoGb3 measured in plasma and urine.
- To establish a baseline for long-term follow up of cellular Gb3 inclusions in skin and renal biopsies.
- To assess viral shedding in various body fluids after systemic administration of FLT190.
- To describe the immune responses to the endogenous product of αGLA following systemic administration of FLT190.

8.3 Exploratory

- 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.
- To investigate the impact of endogenous production of (leukocyte αGLA levels).
- To investigate the impact of endogenous production of αGLA on eGFR.
- To investigate the impact of endogenous production of αGLA on urinary protein and albumin excretion.
- To investigate the impact of endogenous production of αGLA on cardiac parameters.
- To investigate the impact of endogenous production of αGLA on ischemic/hemorrhagic lesions and regions of chronic white matter hyperintensities within the brain.
- To investigate the impact of endogenous production of αGLA on quality of life (QoL).
- To investigate the impact of endogenous production of αGLA on health resource utilization.
- To investigate the impact of endogenous production of αGLA on symptomatic pain.
- To investigate the impact of endogenous production of αGLA on 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.
- To describe the immune response to the AAVS3 capsid proteins following systemic administration of FLT190.

9 Trial Design

9.1 Overall Design

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

Subjects will undergo the screening assessments for up to 18 weeks prior to Study Day 0 (gene therapy infusion).

Treatment-eligible subjects will report 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). Subjects will either attend their local study site if it has been approved for FLT190 infusion, or if not, an alternative infusion study site. On Day 0, FLT190 will be administered as a single dose, slow IV infusion into a peripheral vein, and the subject will remain in the study center for at least 8 hours following the end of the infusion, until the investigator has deemed the subject as fit to be discharged.

Subjects will be required to undergo study evaluations at intervals over the 38-week post-treatment period. These will take place either at the study infusion site or at their local study site. To monitor for shedding of vg sequences, subjects will be required to provide plasma, saliva, urine, stool, and semen samples until three consecutive samples taken at separate visits (at least 1 week apart) are shown to be clear of vg sequences.

This is a first-in-human study and as such an ascending-dose design has been implemented to enable dose evaluation in a step-wise manner. The study will be conducted in 2 parts (Figure 2):

Part 1

Up to 4 dose cohorts of vector will be tested in a dose escalation scheme in 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).

Subjects in the USA will only be enrolled for Cohort 2 and above.

Subjects receiving both ERT and PCT will not be eligible to participate in the study. In Part 1, eligible patients currently receiving ERT will maintain their current treatment until a minimum of 2 consecutive results at least 4 weeks post-dosing show αGLA expression higher than pre-dose trough αGLA expression, after which ERT may be withdrawn. In Part 1, eligible patients currently receiving PCT will have 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 will be observed. Cohorts are planned to include between two and three PTPs. For each subject, safety outcomes (as determined by AE reporting and laboratory tests) and αGLA expression will be evaluated after dosing. Dependent on safety and αGLA expression, a decision will be taken on whether to recruit another subject into the cohort or to proceed to the next dose cohort (Sections 14.3.3 and 14.3.4). Dose escalation will be overseen by an independent DMC.

An interim analysis is planned 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 is to inform the recommended dose for a Phase 3 study in PTPs. When the Part 1 dose escalation is complete (and all patients have at least 12 weeks of data following FLT190

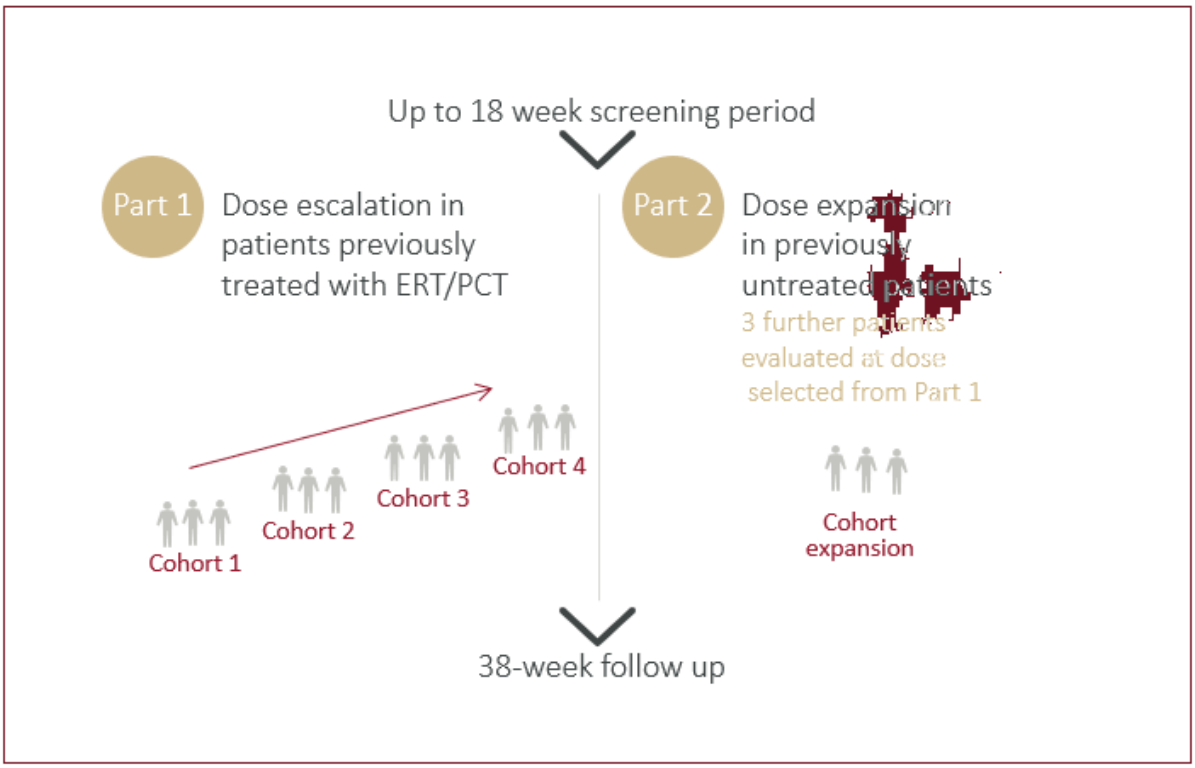
administration), dosing in Part 2 will also be discussed with the DMC to inform the recommended dose for Part 2 in PUPs.

Part 2

A single cohort of three PUPs (PUPs are defined as patients who have never previously received treatment with ERT or PCT [licensed or investigational]) will be enrolled at the dose level selected from Part 1.

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

Figure 2: Study Schematic



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

9.2 Endpoints

9.2.1 Primary Endpoint

- Safety as assessed by the reporting of AEs.

9.2.2 Secondary Endpoints

Safety

- Safety as assessed by reporting of abnormal or change from baseline findings from safety assessments including, laboratory assessments, vital signs, ECG, 24-hour Holter monitor, echocardiogram, cardiac MRI, physical examination, and liver ultrasound.

Efficacy

- Change from baseline in Gb3 and 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 vg in blood, urine, saliva, stool, and semen.

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.

9.2.3 Exploratory Endpoints

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 STtabilization indEX (FASTEX).

- Need for Fabry treatments (including resumption of ERT and 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-Fatigue scale (FACIT-Fatigue).
- Change from baseline in blood plasma immune biomarkers.

Health-related Quality of Life

- Change from baseline in QoL score using the EQ-5D-5L questionnaire at Week 38.

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.

Health Resource Utilization

Change from baseline in the level of health resource utilized by subjects 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.

10 Selection of Subjects

Subject enrolment at a site will only commence once the study has documented ethics committee, competent authority, and local institution approval, and been initiated on behalf of the Sponsor. Subjects may only be enrolled at approved study sites.

10.1 Inclusion Criteria

Subjects will only be eligible to participate in the study if they meet the following inclusion criteria:

1. Adult males, ≥18 years of age with classic Fabry disease.
2. Confirmed diagnosis of classic Fabry disease (including historical documentation of a classic pathological GLA mutation).
3. Plasma αGLA activity at screening (measured by central laboratory activity assay at trough) less than 5% of normal according to the central laboratory reference range.
4. One or more of the characteristic features of classic Fabry disease: neuropathic pain, corneal verticillata, clustered skin angiokeratoma.
5. Estimated glomerular filtration rate (eGFR) ≥60mL/min/1.73m^2 at screening, per serum creatinine, using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.
6. <500 mg/g UPCR in a spot urine sample OR if ≥500 mg/g, <1 g/24 hours of urinary protein (24-hour urine analysis), at screening.
7. Provision of full informed consent and able to comply with all requirements of the study, and willingness to consider participating in the 5-year long-term follow-up.
8. Willingness to practice barrier contraception as specified in the protocol.
9. Lack of neutralizing anti-AAVS3 antibodies using an in vitro transduction inhibition assay within 6 weeks prior to vector administration.
10. For inclusion in Part 1, subjects must have received either a licensed ERT or PCT for at least 12 months prior to FLT190 dosing, at a stable dose (subjects receiving both ERT and PCT will be ineligible). For inclusion in Part 2, subjects must have never previously received treatment with either ERT or PCT (licensed or investigational).
11. Willingness to avoid strenuous exercise during first 3 months after FLT190 dosing.

10.2 Exclusion Criteria

Subjects will not be eligible to participate in the study if they meet any of the following exclusion criteria:

1. Presence of GLA mutations leading to non-classical Fabry disease manifestation and any mutations that have not yet been classified.

   NOTE: Refer to Fabry disease mutation database to determine classic and non-classical mutations. E.g., International Fabry Disease Genotype-Phenotype Database. If there is any uncertainty around the mutation classification discuss with Medical Monitor.

   (http://www.dbfgp.org/dbFgp/fabry/Mutation.html).

2. Prior hypersensitivity or intolerance to ERT.
3. Prior lack of response to ERT.
4. Subjects with a history of chronic kidney disease, stages 3-5 (Kidney Disease: Improving Global Outcomes [KDIGO] 2012 classification), documented in medical records for a minimum of 3 months.
5. Subjects with myocardial fibrosis (≥ 3 segments) identified by MRI at screening.
6. Use of investigational therapy for Fabry disease within 60 days before enrolment. In addition, participation in any other clinical study of an investigational medicinal product (IMP), and/or receiving any other IMP during the study.
7. Persistently elevated ALT or aspartate aminotransferase (AST); or bilirubin >1.5 x upper limit of normal, during screening.
8. Platelet count <100 x 10^9/L.
9. Subjects receiving warfarin or other anticoagulants interfering with the ability to perform renal or skin biopsies, or subjects with a clinically significant bleeding disorder.
10. A history of hepatitis B infection, or a positive serology test at screening for hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb), or negative for hepatitis B surface antibody (HBsAb).
11. A positive serology test at screening for hepatitis C antibody (HCAb) with a positive HCV-RNA load assay, or currently undergoing anti-viral therapy for hepatitis C.
12. A negative test at screening for anti-varicella zoster virus (VZV) IgG.
13. Either history of HIV infection, or a positive serology test at screening for human immunodeficiency virus (HIV).
14. A history of tuberculosis (TB), or a positive screening test for TB. The screening test may be repeated once following an indeterminate result.
15. Subjects who have received live attenuated vaccination within 12 weeks prior to screening or intend to receive such vaccination during the study.
16. Uncontrolled glaucoma, diabetes mellitus, or hypertension.
17. History of any malignancy requiring treatment.
18. History or detection significant arrhythmia on screening assessments with ECG/24-hour Holter monitoring. A significant arrhythmia includes those deemed clinically significant by the investigator e.g. advanced heart block (2^{nd} or 3^{rd} degree), supraventricular or ventricular arrhythmias.
19. Subjects with uncontrolled cardiac failure, unstable angina, myocardial infarction, or other cardiac presentations deemed significant by the investigator in the past 6 months.
20. History of acute myocarditis or presence of acute myocarditis during screening.
21. Prior treatment with any gene therapy medicinal product.
22. Known or suspected intolerance, hypersensitivity or contraindication to gadolinium, tacrolimus and other macrolides, steroids, local anesthetics used for skin or renal biopsies, the IMP and non-investigational medicinal products (NIMPs) or their excipients.

23. Subjects who are assessed as having any contraindications to MRI. Including subjects with ferromagnetic metallic implants, including pacing and defibrillator devices, nerve stimulators and cochlear implants.
24. Subjects who have had a renal transplant.
25. Cytomegalovirus (CMV) Immunoglobulin (Ig)G postive subjects who are CMV polymerase chain reaction (PCR) positive at screening.
26. Current or relevant history of a physical or psychiatric illness or any medical condition that in the opinion of the investigator could affect the subject's safety or interfere with the study assessments.
27. History of substance abuse including alcohol abuse or alcohol dependence.

11 Investigational Medicinal Products and Non-Investigational Medicinal Products

11.1 Name and Description of Investigational Medicinal Product

FLT190 (AAV2/S3-FRE1-GLA-co) is a replication-incompetent single stranded (ss)recombinant AAV vector. The vector is composed of a ss DNA genome packaged in an AAV-derived protein capsid (Section 7.2).

FLT190 is supplied as a sterile Concentrate for Solution for Infusion in 10 mL vials, each vial containing 5 mL extractable volume. The vials are sealed with rubber stoppers and aluminium seals with plastic flip tops. The product is formulated in a phosphate buffered saline solution at neutral pH. The formulation also contains 0.25% w/v recombinant human albumin.

FLT190 Concentrate for Solution for Infusion is stored at ≤-60ºC and must remain frozen until thawed prior to use.

FLT190 Concentrate for Solution for Infusion is classified as an ATIMP and specifically an investigational gene therapy medicinal product.

11.2 Manufacture, Distribution and Storage

11.2.1 Manufacture

FLT190 Drug Substance and Drug Product are manufactured by Henogen SA according to Good Manufacturing Practice and tested according to approved specifications. Testing is performed by Henogen SA or approved Quality Control laboratories. The vg titer is determined as part of the product release testing and is stated on the Certificate of Analysis as well as the product label. The labelled vg titer is used in dose calculations.

11.2.2 Labeling, Secondary Packaging, and Qualified Person Certification

Primary pack labeling, secondary packaging and labeling, and final Qualified Person certification is performed by PCI Pharmaceutical Services.

The Drug Product is shipped from Henogen SA to PCI Pharmaceutical Services (and from PCI Pharmaceutical Services to clinical sites) using companies specializing in cold chain transportation of biopharmaceuticals. Vials are maintained at ≤ -60ºC during shipment. All shipments include temperature monitoring devices so that temperature control during transit can be confirmed.

The FLT190 is labelled in accordance with local regulatory requirements (Annex 13 [EudraLex Volume 4] in the EU). Multilanguage booklet labels are used to ensure label text is provided in the language of each territory where dosing is to be performed.

11.2.3 Distribution

PCI Pharmaceutical Services distribution hubs in the United Kingdom, Ireland and USA are used for shipment of FLT190 Drug Product to sites.

All shipments contain temperature monitoring devices so ensuring the temperature control during shipment can be confirmed.

Administration of FLT190 will be restricted to study sites that have the capacity to store the FLT190 at the specification storage temperature and to prepare and administer the infusion solutions with the required level of control. In addition, as part of the study site selection process, the study site's ability to meet the required safety oversight of the subject will be assessed (e.g. site team emergency response capabilities/procedures and access to the required emergency response equipment/departments).

FLT190 Concentrate for Solution for Infusion is stored frozen at ≤ -60ºC until used for the preparation of the infusion solutions. Depending on the dose, FLT190 may be administered either undiluted or diluted to a reasonable administration volume with commercially obtained 0.9% sodium chloride infusion. Detailed instructions for the preparation of infusion solutions will be provided in the study pharmacy manual.

The appropriate number of vials required for dosing is determined based on the weight of each patient and the allocated dose level.

11.2.4 Receipt, Storage, and Handling

All IMP-related aspects of the study at participating sites are the responsibility of the Investigator, who may delegate this responsibility to the local pharmacist or other appropriately trained personnel. The delegation of duties must be recorded on the Staff Signature and Delegation of Tasks Form.

The FLT190 drug product is received at the investigational site by the Investigator or designee. The temperature log for the shipment is checked to ensure that the vials have remained frozen in transit and the vials are transferred to a restricted access freezer maintaining a temperature of ≤ -60ºC.

Once pre-dose eligibility checks have been confirmed on Day 0, the FLT190 vials are thawed, and the infusion solutions prepared. All procedures will be performed in an appropriate environment such that the sterility of the infusion solutions is assured. FLT190 is administered over the course of 1 to 2 hours. Detailed instructions for the receipt, storage, handling, and preparation of IMP are in the study pharmacy manual.

All local requirements related to activities involving genetically modified organisms will be adhered to.

11.2.5 Accountability and Traceability

There is a system set-up to ensure the traceability of FLT190 from the starting material, through to administration to the participant and destruction or final transfer. A study pharmacy manual and associated Standard Operating Procedures and forms are in place to ensure that the required accountability and traceability data is collected and retained. The requirement for the manufacturer and Investigator/clinical study site(s) to retain their part of the traceability information is set out in the relevant contractual agreements with the Sponsor.

11.3 Name and Description of Non-Investigational Medicinal Product

Immunosuppressants will be provided as commercially available locally.

Prednisolone and Prednisone are used in the same manner and are equally effective. Therefore, the recommended schedule for prednisolone, equally applies to prednisone.

Prednisolone (prednisone), methylprednisolone and tacrolimus are considered to be NIMPs in this study and will be sourced from stocks at the infusion center or local study site.

Investigational sites are responsible for maintaining a system which allows adequate reconstruction of NIMP movements and evaluation of subject compliance.

Risks associated with use of NIMP as described in their Summary of Product Characteristics should be communicated to the subjects by the Investigator.

The usage of immunosuppressants should be recorded on the Immunosuppressant Log in the electronic case report form (eCRF). These details include daily dosage and trough tacrolimus levels.

11.4 Implementation of the Immunosuppressive Regimen

Subjects will be instructed to only take treatment as instructed by the Investigator.

11.4.1 Prophylaxis Regimen

- Following 21 days of observation after vector infusion commence prednisolone at 1mg/kg every day (minimum dose 60 mg/day) for 3 weeks, starting at the Week 3 visit. Round down the dose to the nearest 2.5 mg.
- Commence tacrolimus on the same day as prednisolone.
    - Prescribe and dispense tacrolimus, at a starting dose of 0.2 mg/kg/day. Doses can either be rounded up or down to the nearest 0.5 mg.
    - Tacrolimus trough levels should be measured twice weekly until levels are in the desired range (10-15 ng/mL) and weekly thereafter during tacrolimus treatment.
    - Management guidelines for tacrolimus dosing are included within Appendix 5.
    - CMV testing should be conducted weekly during tacrolimus treatment (if required in response to a subject testing positive for CMV IgG antibodies at screening). In the instance of CMV reactivation management guidelines are included within Appendix 4.
    - Any additional safety monitoring that is required as per local guidelines whilst subjects are receiving prednisolone or tacrolimus treatment should be adhered to. In the event of trauma, serious illness or surgery, risk of adrenal insufficiency should be considered.
    - The investigator can provide bone and gastric protection, and infection prophylaxis (e.g. PCP) as per local practice, as they deem appropriate.
    - Monitoring LFTs and plasma αGLA expression while on prophylactic immunosuppression should be conducted in accordance with Table 1.
- Prednisolone taper:
    - Commence prednisolone taper when tacrolimus trough levels are in the desired range (approximately 10-15 ng/mL, on two consecutive occasions) and after a minimum of 21 days of prednisolone.

- Decrease prednisolone by 15% of the initial dose every week until 10 mg is reached (rounding down the dose to the nearest 5 mg at each increment).
- Continue 10 mg for three weeks then reduce dose to 5 mg for two weeks followed by 2.5 mg for a week then stop prednisolone.
- NOTE: The time at which the prednisolone taper ceases will vary based on the starting dose and the time when the taper was initiated.

- Tacrolimus taper:
  - At the end of week 10 reduce the dose of tacrolimus to maintain levels between 5-10 ng/mL for two weeks and then reduce the dose further to maintain levels between 1-5 ng/mL for another two weeks before stopping.
  - The tacrolimus taper may begin earlier, between the end of weeks 6 and 10 or the taper may be omitted to reduce the exposure to tacrolimus. In consultation with the Medical Monitor, this decision will be dependent on the timing of potential episodes of early transaminitis, or any early upward trends in ALT indicative of potential transaminitis.
- In individual subjects the immunosuppressant regimen maybe adapted based on medical need. This will be considered in consultation with the Medical Monitor.

If an elevation in ALT / transaminitis is observed, follow the instructions below.

11.4.2 Reactive Regimen (in Response to ALT Elevation)

- At all times, Investigators should be vigilant of a downward trend in αGLA expression, which alongside an elevation in ALT, may be an early indicator of transaminitis.
- When a subject has an elevation in ALT or the trend of liver function tests (LFTs) and/or αGLA levels suggest potential transaminitis or a lack of response to oral prednisolone/tacrolimus, consider increasing the frequency of LFT testing or urgent evaluation and/or escalation of immune suppression:
  - An increase in ALT >1.5x baseline, even if this level does not exceed the upper limit of normal, should trigger a repeat test of ALT within 24 hours
  - An increase in ALT above the upper limit of normal, or with an upward trend on repeat testing, or other signs of potential transaminitis should trigger urgent evaluation
  - Evaluate to exclude other causes of increased liver enzymes. Assessments to consider include:
    - Consider recent alcohol, drug, and recreational drug intake.
    - Exclude history of recent intense physical exercise.
    - Blood tests: FBC, CRP, ALT, AST, ALP, GGT, LDH, bilirubin, troponin, CK.
    - Infectious screen, including viral serology.
    - Hepato-biliary imaging.

Assume it is vector associated transaminitis after reasonable attempts to exclude other causes.

- Record/report the event accordingly in the source documentation and eCRF. See Section 15 for reporting of AEs/serious adverse events (SAEs)/adverse events of special interest (AESIs)
- Contact your CRO Medical Monitor and Sponsor immediately.
- If possible, collect a research plasma and peripheral blood mononuclear cells (PBMC) research sample as soon as possible after initial evaluation and prior to initiation of escalation of immune suppression, but treatment must not be delayed. If possible, continue to collect weekly research plasma and PBMC samples until ALT levels return to within normal range. Research samples routinely collected as part of the Schedule of Assessments may be used, and PBMC samples should only be collected if the subject attends the study site. Instructions for the collection and processing of research plasma and PBMC samples are in the study laboratory manual.
- Commence urgent escalation of immune suppression to treat vector associated transaminitis

    1. If the subject is receiving the prophylactic immune suppression regimen described in Section 11.4.1 when vector associated transaminitis occurs:
        a. Administer methylprednisolone 1g intravenously daily for 3 days.
        b. Check tacrolimus trough levels and if required adjust dose to ensure desired levels. If tacrolimus is not currently in use, it may be initiated after consultation with the medical monitor.
        c. Following methylprednisolone prednisolone at 1 mg/kg/day may be initiated after consultation with the medical monitor (minimum dose 60 mg/day).
    2. If the subject has completed the prophylactic immune suppression regimen when vector associated transaminitis occurs:
        a. Administer IV methylprednisolone 1 g intravenously daily for 3 days.
        b. Tacrolimus at 0.2 mg/kg/day may be initiated after consultation with the medical monitor. Doses can either be rounded up or down to the nearest 0.5 mg. Tacrolimus trough levels should be measured twice weekly until levels are in the desired range (10-15 ng/mL) and weekly thereafter during tacrolimus treatment.
        c. Prednisolone at 1 mg/kg/day (minimum dose 60 mg/day) may be initiated after methylprednisolone after consultation with the medical monitor.

- Monitor LFTs daily initially to establish response to treatment.
- Prednisolone taper:
  - Commence prednisolone taper when tacrolimus trough levels are in the desired range (approximately 10-15 ng/mL, on two consecutive occasions), LFTs are in the normal range (on 2 consecutive occasions) and after a minimum of 21 days of prednisolone.
  - Decrease prednisolone by 15% of the initial dose every week until 10 mg is reached (rounding down the dose to the nearest 5 mg at each increment).
  - Continue 10 mg for two weeks then reduce dose to 5 mg for one week followed by 2.5 mg for a week then stop prednisolone.
- Tacrolimus taper:
  - Commence tacrolimus taper once LFTs are in the normal range, on 2 consecutive occasions, and tacrolimus trough levels are in the therapeutic range, on 2 consecutive occasions.
  - Reduce the dose of tacrolimus to maintain levels between 5-10 ng/mL for two weeks and then reduce the dose further to maintain levels between 1-5 ng/mL for another two weeks before stopping.
  - The tacrolimus taper may be forgone or begin earlier or later depending on the response to escalation of immune suppression, degree of transaminitis, and in consultation with the Medical Monitor.
- Management guidelines for tacrolimus dosing are contained within Appendix 5.
- Cytomegalovirus testing should be conducted weekly during tacrolimus treatment (if required in response to a subject testing positive for CMV IgG antibodies at screening). In the instance of CMV reactivation management guidelines are included within Appendix 4.
- Any additional safety monitoring that is required as per local guidelines whilst subjects are receiving prednisolone and tacrolimus treatment should be adhered to. In the event of trauma, serious illness or surgery, risk of adrenal insufficiency should be considered.
- The Investigator can provide bone and gastric protection, and infection prophylaxis (e.g. PCP) as per local practice, as they deem appropriate.
- LFTs and αGLA activity level should be monitored regularly two or three times a week whilst the subject is on immunosuppressant therapy and should continue for two weeks following cessation of immunosuppressants.

- The Investigator, in consultation with the Sponsor, may use clinical judgement to assess the appropriate course of treatment and monitoring in response to ANY rise in ALT or if any other cause (suspected or confirmed) is identified.
- In individual subjects the immunosuppressant regimen may be adapted based on medical need. This will be considered in consultation with the Medical Monitor.

Where there is contact or consultation with the CRO Medical Monitor, the Sponsor should be kept informed.

AESIs should be reported to the Sponsor within 24 hours, see Section 15.2.10 for further details relating to the definition of AESI and their reporting requirements.

12 Concomitant Medication

All non-study treatment received within 30 days prior to initial informed consent and for the duration of the study must be recorded on the appropriate eCRF page. Concomitant medication includes prescription and non-prescription medication and herbal treatments.

12.1 Prior Treatment

Prior treatment includes all treatment received from 30 days prior to the initial informed consent up until the time of FLT190 infusion. Prior treatment information must be recorded on the appropriate eCRF page.

12.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the date of investigational product infusion and the Week 38/end of study (EOS) visit, inclusive. All concomitant treatments information must be recorded on the appropriate eCRF page.

Subjects should be instructed not to start taking any new medications, including non-prescription drugs and herbal preparations, unless they have received permission from the Investigator, time allowing, i.e. investigator permission not required for emergency medical care.

Subjects with either a medical history or evidence at screening of equivalent of ≥500 mg/24 hours of urinary protein will be required to either commence or continue treatment with a renin-angiotensin aldosterone system blocker (RASB), unless contraindicated. See Appendix 2 for RASB treatment guidance.

Other therapy considered necessary for the subject's welfare may be given at the discretion of the Investigator. All such therapy must be recorded in the eCRF. No other advanced therapy medicinal product /IMP may be used concomitantly with the study treatment. The subjects are not allowed to participate concurrently in another clinical study.

Any systemic immune-modifying medication other than immune suppression prescribed in accordance with this protocol should be discussed with the Medical Monitor. Local or topical steroids, e.g. use of inhaled corticosteroids to manage chronic respiratory conditions are permitted.

In the event of an emergency, any needed medications may be prescribed without prior approval, but the Medical Monitor must be notified of the use of any excluded medications immediately thereafter.

12.3 Fabry Disease Therapy

Prior use, recommencement, or initiation of Fabry disease therapy, including ERT, PCT or any other medicinal product that may impact αGLA levels should be documented in the Fabry Treatment eCRF page.

12.3.1 Withdrawal from Current Therapy

In Part 1, eligible patients currently receiving ERT will maintain their current treatment until a minimum of 2 consecutive results at least 4 weeks post-dosing show αGLA expression higher

than pre-dose trough αGLA expression, after which ERT may be withdrawn. In Part 1, eligible patients currently receiving PCT will have 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 will be observed.

12.3.2 Recommencing Previous Therapy (Part 1 - PTPs)

If either the predicted AUC endogenous production of αGLA is projected to fall short of levels achieved by existing ERT/PCT, or if there is clinical evidence of a lack of response (such as <5% normal plasma αGLA according to the central laboratory reference range, or clinical signs or symptoms previously reported related to Fabry disease that improved while on ERT/PCT), the Investigator may restart ERT/PCT treatment after discussion with the Sponsor.

12.3.3 Initiating Fabry Disease Treatment (Part 2 - PUPs)

If either the predicted AUC endogenous production of αGLA is projected to fall short of levels achieved by existing ERT/PCT), or if there is clinical evidence of a lack of response (such as <5% normal plasma αGLA according to the central laboratory reference range, or clinical signs or symptoms previously reported related to Fabry disease), the Investigator may start ERT/PCT after discussion with the Sponsor. Where indicated, this will be done in line with local guidelines for treatment of Fabry disease.

13 Study Procedures

13.1 Study Schedule

13.1.1 Screening

The following study-specific procedures will be carried out after the informed consent form (ICF) is signed to assess the patient's eligibility. Screening evaluations will be performed at the study site. Screening evaluations may take place over a number of visits as necessary to complete all assessments. Screening laboratory assessments can be repeated on one further occasion if the initial test result falls outside of the protocol eligibility criteria. A window of up to 18 weeks (from initial consent to Day 0) is allowed for screening. Subjects may be rescreened should this time window elapse.

- Demography and medical history.
- Fabry disease, ERT, and PCT history.
- Prior and concomitant medication.
- AEs will be recorded during the screening period from the time of initial consent.
- Physical examination including measurement of the subject's height, weight, waist circumference, hip circumference, neck circumference, and bioimpedance.
- Vital signs (pulse, blood pressure, respiration rate, and temperature).
- 12-lead ECG.
- 24-hour Holter monitor.
- Echocardiogram.
- Liver ultrasound scan.
- Subjects will be asked to complete an electronic diary during the screening period, answering questions relating to:
    - Pain during the last 24 hours – on a daily basis for the screening period (SFBPI).
    - Fatigue and GI symptoms during the last 7 days – every 7 days (FACIT-Fatigue and GSRS).

  Subjects will be issued with instructions on electronic diary completion.

  If screening will be conducted over an extended period of time, there can be a temporary discontinuation of electronic diary completion following discussion with the Sponsor/Medical Monitor. At a minimum, subjects should complete the electronic diary during the initial ERT/PCT cycles during the screening period and during the 7 days prior to FLT190 dosing.
- Cardiac and brain MRI with weight-adjusted gadolinium-based contrast agent.
- Fabry disease severity will be scored using the MSSI and FASTEX (FASTEX should be completed following the cardiac MRI).

- Skin and renal biopsy (details in the study skin and renal biopsy manual) (only conducted when all eligibility-determining screening assessments have been completed and the subject has been confirmed as being eligible to participate in the study).
- QoL will be evaluated using the EQ-5D-5L questionnaire.
- Health resource utilization will be documented, including during the screening period and for the 6-month period prior to the initial screening visit.
- Blood sample to evaluate troponin (local laboratory analysis); I or T per available local assay.
- Blood samples will be collected to evaluate (central laboratory analysis):
    - Anti-AAVS3 neutralizing antibody test. Note: A blood sample drawn within 6 weeks of dosing must be confirmed as negative before a subject can be considered for dosing. It is acceptable to take repeat samples during the screening period, as necessary.
    - Hematology, chemistry (including LFTs, hs troponin-T and NT-proBNP), TB screen, and viral serology.*
    - αGLA plasma activity and concentration levels.**
    - αGLA leukocyte activity.**
    - Plasma Gb3 and lysoGb3.
    - αGLA immune response.

    * Blood samples will be taken for serology including hepatitis B (HBsAg, HbcAb, HbsAb), hepatitis C (HCAb and HC PCR; HC PCR only indicated for subjects with positive HCAb screen), HIV 1 and 2 antibodies, CMV (CMV IgG test and CMV PCR; CMV PCR only indicated for subjects with positive CMV IgG screen), and VZV IgG.

    ** In Part 1 (PTPs) the αGLA blood sample is to be collected within 48 hours prior to the subject's next scheduled ERT dosing, to ensure the αGLA activity level is measured at trough.
- Blood sample will be collected to evaluate (central or local laboratory analysis):
    - INR, PTT, and hemoglobin (prior to the renal/skin biopsies).
- Urine samples will be collected to evaluate:
    - Gb3 and lysoGb3 clearance.
    - Urinalysis (prior to the renal biopsy).
    - Spot urine to evaluate protein/creatinine and albumin/creatinine ratios. Subjects will be asked to provide a first morning, void/mid-stream sample on the day of the visit (and for 2 days prior).
    - If a spot urine protein/creatinine ratio is >500mg/g, a 24-hour urine collection will be obtained to measure 24-hour total protein and albumin excretion and creatinine clearance. Urine protein/creatinine ratio and urine albumin/creatinine ratio will also be measured on this 24-hour urine collection.

- eGFR will be derived.

ALT levels will guide treatment for transaminitis during the study and alcohol intake can cause ALT levels to rise, making it difficult to determine whether transaminitis is alcohol or vector associated. As such, a subject's alcohol intake for the course of the study should also be discussed at the screening visit with a recommendation to moderate their intake for the duration of the study. Further, subjects will be expected to abstain from alcohol and strenuous exercise from 1 week prior to vector infusion and for approximately 3 months following vector infusion. Discussion around a subject's alcohol intake should be ongoing throughout the study.

Subjects should be up to date with local vaccination policy (e.g. seasonal flu vaccination).

13.1.2 Subject Re-screening

Subjects may be rescreened should the screening window elapse. Subjects that are rescreened into the study will receive a new subject number and will be required to reconsent for participation in the study.

As part of the subject rescreen study visit the assessments must be repeated as described in Section 13.1.1 to ensure the subject remains eligible for the study.

Renal and skin biopsies, brain MRI, liver ultrasound, MSSI, and FASTEX only need to be repeated if more than 6 months has been exceeded since the assessment was completed during the subject's initial screening period and the subject's planned dosing date.

Subjects will be required to complete pain, fatigue, and GI electronic diaries during the rescreening period up until Day -1.

13.1.3 Pre-infusion Infection Risk Considerations

Subjects will receive a course of prophylactic immune suppression consisting of prednisolone and tacrolimus starting from week 3 after dosing with FLT190. Therefore, prior to infusion, measures to minimize infection risk should be considered.

Subjects should be up to date with local vaccination policy (e.g. seasonal flu vaccinations).

Avoid live vaccines while on tacrolimus. Subjects who have received live attenuated vaccination within 12 weeks prior to screening or intend to receive such vaccination during the study are excluded.

Perform testing for SARS-CoV-2 (COVID-19) in line with current local practice, such as 1 or 2 nasopharyngeal negative swabs and absence of symptoms within 72 hours of dosing. Any signs or symptoms in the two weeks prior to dosing should be discussed with the medical monitor prior to dosing. Any confirmed COVID-19 positive tests, prior to or after FLT190 infusion, should be reported to the Sponsor within 24 hours.

13.1.4 Vector Infusion (Day -1 to Day +2)

Vector infusion will only occur once all screening assessments have been conducted and the subject is deemed to be eligible for the study. Eligible subjects will either attend their local study site if it has been approved for FLT190 infusion, or if not, an alternative infusion study site, prior to the day of vector infusion (Day -1). From Day -1 to Day +2, local safety laboratory analysis for blood samples will be utilized for management of subjects to ensure rapid

turnaround of clinical results (Section 13.4.3). In some cases, duplicate blood samples will be drawn for central analysis as detailed in the list of procedures below. The following procedures will be undertaken:

Day -1

Day -1 assessments may be conducted as early as Day -3 for logistical reasons if required.

- Physical examination (including confirmation of weight, waist circumference, hip circumference, neck circumference, and bioimpedance).
- Vital signs (pulse, blood pressure, respiration rate, and temperature).
- 12-lead ECG.
- AE and concomitant medication status.
- Blood samples will be collected to evaluate (local laboratory analysis):
    - Hematology, chemistry, and LFTs: chemistry differentials to include sodium, potassium, phosphate, blood urea nitrogen, serum creatinine, and C-reactive protein; LFTs to include albumin, alkaline phosphatase, direct/indirect and total bilirubin, ALT, and AST.
- Blood samples will be collected to evaluate (central laboratory analysis):
    - Hematology, chemistry, and LFTs (including amylase and hs troponin-T levels).
    - AAVS3 immune response (antibodies and T-cell responses).
    - αGLA immune response.
    - Anti-AAVS3 neutralizing antibodies – to retrospectively check status immediately prior to dosing.
    - Gb3 and lysoGb3.
    - Research samples:
        - Mononuclear cells.
        - Biomarkers and plasma.
- Serum COVID-19 test will be performed as per local practice within 72 hours prior to patient dosing (Section 13.1.3).
- Urine samples will be collected to evaluate:
    - Gb3 and lysoGb3.
    - Spot urine to evaluate protein/creatinine and albumin/creatinine ratios.
    - Future research.
- eGFR will be derived.
- Plasma, saliva, stool, urine, and semen samples will be taken to evaluate vector shedding.
- Completion of SFBPI, GSRS, and FACIT-Fatigue questionnaires.

Day 0

- Intravenous catheter insertion into a suitable peripheral vein (e.g., the median cubital vein).

- The IV catheter will be flushed with saline.
- The vector will be thawed and diluted in accordance with instructions in the study pharmacy manual.
- Prepared drug will be kept at room temperature prior to administration.
- At approximately 1 hour prior to vector infusion, the investigator or designee will assess the subject's vital signs and blood samples will be collected to analyze:
    - Hematology, chemistry, and LFTs (local laboratory analysis): chemistry differentials to include sodium, potassium, phosphate, blood urea nitrogen, serum creatinine, and C-reactive protein; LFTs to include albumin, alkaline phosphatase, direct/indirect and total bilirubin, ALT, and AST).
    - αGLA plasma activity and concentration levels, and αGLA leukocyte activity (central laboratory analysis).
- The prepared drug containing the calculated vector dose will be infused through the catheter using an appropriate administration set and infusion pump, according to the study pharmacy manual.
- Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored at 15-minute intervals (± 5 min) from the start to completion of infusion and then hourly from the end of infusion (± 10 min) i.e. +1, +2, 3, 4, 5 and 6 hours. Further measurements will be taken at +8 hours (± 15 min) and every 2 hours, if deemed necessary by the investigator.
- AEs and concomitant medications will be monitored at -1 hour, 0 hour, and at 15-minute intervals (± 5 min) from the start to completion of infusion and then hourly from the end of infusion (± 10 min) i.e. +1, +2, 3, 4, 5 and 6 hours. Further measurements will be taken at +8 hours (± 15 min) and every 2 hours if deemed necessary by the investigator.
- At approximately 6 hours from the end of the FLT infusion, blood samples will be collected to evaluate:
    - Hematology, chemistry, and LFTs (local laboratory analysis): chemistry to include sodium, potassium, phosphate, blood urea nitrogen, serum creatinine, and C-reactive protein; LFTs to include albumin, alkaline phosphatase, direct/indirect and total bilirubin, ALT, and AST).
- On completion of the infusion, the catheter will again be flushed with saline.
- Subjects will remain at the infusion center for at least 8 hours post-infusion to observe for any immediate toxicity of the procedure. If the vital signs are stable, the catheter will be removed prior to subject discharge from the center.
- Subjects may be discharged from the study site. All subjects will receive a medical alert card.

Days +1 and +2

The study visits procedures scheduled on Days +1 and +2 following infusion will be performed at the infusion center where the subject received their vector infusion by the investigator or designee.

The following procedures will be performed at each visit (unless otherwise specified):

- Physical examination.
- Vital signs (pulse, blood pressure, respiration rate, and temperature).
- 12-lead ECG.
- AE and concomitant medication status.
- Blood samples will be collected to evaluate:
    - Hematology, chemistry, and LFTs (local laboratory analysis): chemistry differentials to include sodium, potassium, phosphate, blood urea nitrogen, serum creatinine, and C-reactive protein; LFTs to include albumin, alkaline phosphatase, direct/indirect and total bilirubin, ALT, and AST.
    - Hematology, chemistry, and LFTs (central laboratory analysis: Day +2 only).
- Plasma, saliva, urine, stool, and semen samples will be to evaluate vector shedding (central laboratory analysis; Day +2 only).

13.1.5 Follow-up Visit (Day 5)

The following study visit procedures scheduled on Day +5 following infusion will be performed at the subject's local study site.

- Vital signs (pulse, blood pressure, respiration rate, and temperature).
- 12-lead ECG.
- AE and concomitant medication status.
- Blood samples to evaluate:
    - LFTs (local laboratory analysis): to include albumin, alkaline phosphatase, direct/indirect and total bilirubin, ALT, and AST.
    - αGLA expression - plasma αGLA activity and concentration (central laboratory analysis).
- Plasma, saliva, urine, stool, and semen samples will be taken to evaluate vector shedding (central laboratory analysis).

13.2 Follow-up Visits (Weeks 1-37)

Weeks 1-32 evaluations will be performed at the patient's local study site, or, at the investigator's discretion, may be conducted at home with a visiting nurse performing assessments and sample collection. In Part 1, eligible patients currently receiving ERT will maintain their current treatment until a minimum of 2 consecutive results at least 4 weeks post-dosing show αGLA expression higher than pre-dose trough αGLA expression, after which ERT

may be withdrawn. In Part 1, eligible patients currently receiving PCT will have 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 will be observed.

13.2.1 Week 1-32 Assessments

The following assessments will be conducted at each visit (or as specified):

- AE and concomitant medication status.
- Physical examination.
- Vital signs (pulse, blood pressure, respiratory rate, and temperature).
- 12-lead ECG.
- 24-hour Holter monitor (Weeks 4 and 10 only).
- Echocardiogram (Weeks 4, 8, 12 and 16 only).
- Health resource utilization.
- Subjects will be asked to complete an electronic diary commencing at Weeks 4, 12, and 24, completing the following questionnaires:
    - SFBPI during the last 24 hours – On a daily basis for 7 consecutive days commencing at the start of each specified week.
    - FACIT-Fatigue and GSRS completed at the end of the specified weeks.

  Subjects will be issued with instructions on electronic diary completion.
- Blood samples will be collected to evaluate (local laboratory analysis):
    - LFTs (to include albumin, alkaline phosphatase, direct/indirect and total bilirubin, ALT, and AST). Week 1 to the end of Week 9 and Week 27 to the end of Week 37: testing to be performed twice weekly (including the testing as part of the scheduled study visit, initially commencing at. The tests should be as evenly spaced through the week as possible, for example: Monday and Thursday, or Tuesday and Friday. Week 10 to the end of Week 26: testing to be performed three times weekly (including the testing as part of the scheduled study visit) commencing. The tests should be as evenly spaced through the week as possible, for example Monday, Wednesday, and Friday.
    - Troponin (I or T) will also be collected locally based on available local assay at each visit up to and including Week 37).
- Blood samples will be collected to evaluate (central laboratory analysis):
    - Hematology, chemistry (including hs troponin-T and NT-proBNP), and LFTs.
    - αGLA expression – plasma αGLA activity and concentration levels. Week 1 to the end of Week 9 and Week 27 to the end of Week 37: testing to be performed twice weekly (including the testing as part of the scheduled study visit, initially commencing at. The tests should be as evenly spaced through the week as possible, for example: Monday and Thursday, or Tuesday and Friday. Week 10 to the end of Week 26: testing to be performed three times weekly (including

the testing as part of the scheduled study visit) commencing. The tests should be as evenly spaced through the week as possible, for example Monday, Wednesday, and Friday.

- αGLA leukocyte activity levels (Weeks 4, 12, and 24).
- αGLA immune response (Weeks 1, 2, 3, 6, 12, and 24).
- Plasma Gb3 and lysoGb3 (Part 1) (Weeks 2, 4, 6, 8, 12, 16, 20, 24, 28, and 32).
- Plasma Gb3 and lysoGb3 (Part 2) (Weeks 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, and 32).
- AAVS3 immune response (antibodies) (Weeks 1, 2, 6, 12, 24).
- AAVS3 immune response (T-cell responses).
- Research samples:
  - Mononuclear cells (Weeks 4 and 12).
  - Biomarker and plasma.
  - Serum.

- Urine samples will be collected to evaluate:
  - Gb3 and lysoGb3 (Part 1) (Weeks 2, 4, 6, 8, 12, 16, 20, 24, 28, and 32).
  - Gb3 and lysoGb3 (Part 2) (Weeks 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, and 32).
  - Spot urine to evaluate protein/creatinine and albumin/creatinine ratios – Weeks 4, 8, 12, 16, 20, 24, 28, and 32.*

  * Subjects will be asked to provide a first morning, void/mid-stream sample on the day of the visit (and for 2 days prior).
- eGFR will be derived at Weeks 4, 8, 12, 16, 20, 24, 28, and 32.
- For patients positive for CMV IgG at screening, weekly CMV PCR testing should be conducted for the duration of the immunosuppressant regimen to monitor for reactivation. Management guidelines in the case of CMV reactivation can be found in Appendix 4.
- Commencing at the Week 3 visit, subjects will receive a tapering dose of immunosuppressants (Section 11.4). From Week 3 tacrolimus trough levels should be measured twice weekly until desired levels are established and weekly thereafter in conjunction with immunosuppressant dosing. Management guidelines for tacrolimus dosing can be found in Appendix 5.
- Plasma, saliva, urine, stool, and semen samples will be collected to evaluate vector shedding. Samples will be collected three times within 7-10 days of vector infusion and then weekly until the results from all samples (all matrices) taken at three consecutive visits (at least 1 week apart) are negative. Semen samples may be omitted at the discretion of the PI for religious or other personal reasons expressed by the subject.

13.2.2 Continuance of Subject Care During Exceptional Circumstances (e.g. COVID-19)

In exceptional circumstances, such as restrictions in movement due to COVID-19 where the subject is unable to attend the study site for scheduled visits, to maintain patient safety and ensure continued care the assessments may be conducted at an alternative location (e.g. patient's home, or local medical facility) either by study site personnel, local doctor, or by a home nursing vendor. Implementation of any such arrangements must be agreed in advance by the Sponsor.

13.3 Week 38/End-of-Study Visit

The following assessments will be conducted at Week 38. Should the subject discontinue the study at an earlier time point, every attempt should be made to conduct the Week 38 assessments to ensure adequate safety follow-up.

- AE and concomitant medication status.
- Physical examination.
- Vital signs (pulse, blood pressure, respiration rate, and temperature).
- 12-lead ECG.
- 24-Holter monitor.
- Liver ultrasound scan.
- Subjects will be asked to complete an electronic diary commencing at Week 37 for 7 consecutive days, completing the following questionnaires:
    - SFBPI during the last 24 hours – on a daily basis.
    - FACIT-Fatigue and GSRS during the last 7 days – On the 7th day of the diary completion.
- Cardiac and brain MRI with weight-adjusted gadolinium-based contrast agent.
- Fabry disease severity will be scored using the MSSI and FASTEX.

  Study sites will contact subjects at the beginning of Week 37 to remind them to complete the questionnaires during Week 37.
- QoL will be evaluated using the EQ-5D-5L questionnaire.
- Health resource utilization will be evaluated.
- Blood samples will be collected to evaluate (local laboratory analysis):
    - LFTs (to include albumin, alkaline phosphatase, direct/indirect and total bilirubin, ALT, and AST).
    - Troponin (I or T) will also be collected locally based on available local assay.
- Blood samples will be collected to evaluate (central laboratory analysis):
    - Hematology, chemistry (including hs troponin-T, NT-proBNP, and LFTs).
    - αGLA plasma activity and concentration levels.

- αGLA leukocyte activity level.
- αGLA immune response.
- AAVS3 immune response (antibodies and T-cell responses).
- Gb3 and lysoGb3 plasma samples (Parts 1 and 2).
- Research samples:
  - Mononuclear cells.
  - Biomarker and research plasma.
  - Serum.

- Urine samples will be collected to evaluate:
  - Gb3 and lysoGb3 (Parts 1 and 2).
  - Spot urine to evaluate protein/creatinine and albumin/creatinine ratios*.
  - Future research.

* Subject will be asked to provide a first morning void/mid-stream sample on the day of the visit (and for 2 days prior).

- eGFR will be derived.
- Plasma, saliva, urine, stool, and semen samples will be taken for evaluation of vector shedding; only if there have not been three prior consecutive negative specimens taken at separate visits (at least 1 week apart). Semen samples may be omitted at the discretion of the PI for religious or other personal reasons expressed by the subject.

On completion of the study, subjects will be followed under a separate long-term follow-up protocol for at least a total of 5 years after dosing.

13.4 Study Procedures/Evaluations

All study assessments will be conducted in accordance with Table 1 and Table 2.

For all laboratory samples, the procedures for collection, processing, storing, and transporting to the central laboratory are described in the study laboratory manual.

13.4.1 Demography and Baseline Assessments

Informed Consent Procedure

The informed consent process will be conducted at the screening visit.

It is the responsibility of the investigator, or a suitably qualified co-investigator delegated by the investigator, to obtain written informed consent from each subject before any study related procedures are undertaken. The Investigator or designee will explain the aims, methods anticipated benefits, and potential hazards of the studies, that the subjects are under no obligation to participate and that they can withdraw at any time, without having to give a reason.

A copy of the signed ICF will be given to the subject. The original signed ICF will be retained at the study site and a copy placed in the medical notes.

As part of the consent process, subjects will be informed that in the event of death, no matter what cause, permission for an autopsy will be requested of their families. Subjects will be asked to advise their families of this request and of its scientific and medical importance should they choose to participate.

Subject Identification Code

On enrolment to the study, the subject will be given a unique subject identification code consisting of a 2-digit country number (e.g., 44), a 2-digit site number (e.g., 03), a 1-digit study number (for this study 1), and a 3-digit subject number (e.g., 004). Using these specifications, the fourth subject enrolled at site 03 in the United Kingdom (country code 44) will have the unique subject identification code 44031004. Allocation of the subject identification code will be handled through the eCRF.

Demography

The following demographic information will be collected for each subject at the screening visit:

- Year of birth
- Race
- Ethnicity
- Educational/career status

Medical History

A 5-year medical history should be taken during the screening period. Details of any clinically relevant abnormalities should be noted on the eCRF.

Fabry Disease and ERT/PCT History

The subject's Fabry medical history and ERT/PCT history (if applicable) will be recorded in the eCRF, including the following information:

- Age at diagnosis
- Clinical symptoms (including onset dates)
- GLA genotype
- ERT history and any associated infusion reactions (Part 1 only)

Freeline Anti-AAVS3 Neutralizing Antibody Test

During the screening period, a blood sample will be taken from the subject to determine the presence or absence of neutralizing antibodies to the AAVS3 serotype. A negative result within 6 weeks of dosing is required to confirm eligibility. It is acceptable to take repeat samples during the screening period.

Tuberculosis Screen

A blood sample will be taken at screening to assess TB status. A positive test or repeated indeterminate tests will prevent the subject from enrolling in this study and a follow up discussion with a respiratory specialist should be organized for further assessment.

Serology Screening

A blood sample will be taken at screening to assess the following:

- Hepatitis B status (HBsAg, HBcAb, HBsAb)
- Hepatitis C status (HCAb and HC PCR) (HC PCR only indicated for subjects with a positive HCAb screen)
- HIV 1 and 2 (anti-HIV1/2) antibodies
- CMV IgG antibodies and CMV PCR (CMV PCR only indicated for subjects with a positive CMV IgG screen)
- VZV IgG

Urine Protein and 24-hour Urine Collection

A spot urine sample will be analyzed at the screening visit for protein/creatinine and albumin/creatinine levels. If the protein/creatinine ratio is >500 mg/g (approximate to 50 mg/mmol), a 24-hour urine collection will be obtained to measure 24-hour total protein and albumin excretion and creatinine clearance. Urine protein/creatinine ratio and urine albumin/creatinine ratio will also be measured on this 24-hour urine collection.

This assessment is required to ensure subjects meet the inclusion criteria of having <1 g urinary protein per 24 hours.

13.4.2 Efficacy and Pharmacokinetics

αGLA Expression - Plasma αGLA Activity and Concentration

A bloodspot will be created by spotting whole blood from venepuncture collection onto a dried blood spot test card. The remaining blood will be processed for plasma and leukocyte αGLA activity and plasma αGLA concentration. Samples may also be assessed for other related biomarkers e.g. β-galactosidase and hexosaminidase. Samples will be analyzed at a central laboratory.

αGLA Leukocyte Activity Analysis

Blood samples will be drawn to assess leukocyte αGLA level. Samples may also be assessed for other related biomarkers e.g. β-galactosidase and hexosaminidase. Samples will be analyzed at a central laboratory.

Gb3 and LysoGb3

A bloodspot will be created by spotting whole blood from venepuncture collection onto a dried blood spot test card. The remaining blood will be processed for analysis of Gb3 and lysoGb3 clearance. Samples will be analyzed at a central laboratory.

Gb3 and LysoGb3 Cellular Inclusion

Skin and renal biopsy samples will be collected at screening to establish a baseline for long-term follow up of cellular Gb3 inclusions. De-identified tissue samples will be analyzed at two collaborating central laboratories by masked observers using quantitative unbiased morphometric methods. Skin and renal biopsy methods will be described in the study skin and renal biopsy manual.

Blood pressure and safety laboratory assessments including INR, PTT, hemoglobin, and platelet count will be analyzed pre-biopsy to ensure it is safe for the subject to undergo the procedure. A urinalysis will also be performed to confirm absence of infection.

The skin and renal biopsies will be followed up with additional biopsies being collected as part of the 5-year post-dosing long-term follow-up study.

MSSI and FASTEX

Fabry disease severity will be assess using the MSSI and FASTEX.

13.4.3 Safety

Physical Examination

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.

The subject's height (screening only) and weight will also be measured. Waist circumference (cm), hip circumference (cm), neck circumference (cm) and bioimpedance will be measured at screening and Day -1 only.

Laboratory Safety Assessments

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

- Hematology: complete blood count with differential, platelet count.

  INR, PTT, platelets, and hemoglobin will be measured at the screening visit prior to renal/skin biopsies. This analysis may be performed by the local or central laboratory.

- Chemistry: sodium, potassium, chloride, phosphate, CO_2, glucose, blood urea nitrogen, serum creatinine, cystatin C, C-reactive protein. Liver function tests: albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, ALT, AST, and total protein.

- Urinalysis – A midstream, clean-catch urine specimen will be collected (prior to the renal biopsy) for dipstick analysis to confirm absence of infection. Analysis may be conducted by the local or central laboratory. Further follow-up analysis can be conducted to confirm infection as a result of a positive dipstick analysis. If there is an explanation for the positive dipstick result, it should be recorded, and there is no need to perform further local confirmatory analysis.

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

- Hematology: complete blood count with differential.
- Chemistry: sodium, potassium, phosphate, blood urea nitrogen, serum creatinine, C-reactive protein, and troponin (I or T) depending on local assay availability.
- Liver Function Tests: albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, ALT, and AST.

Blood samples for tacrolimus levels and CMV testing will be analyzed locally. On days where other clinical assessments are not required these samples may be taken at study site or an

alternative location (e.g. patient's home). Management guidelines in the case of CMV reactivation can be found in Appendix 4. Management guidelines for tacrolimus dosing can be found in Appendix 5.

If immunosuppressants are implemented at any other time, monitoring for tacrolimus levels and CMV testing (if required) should be undertaken in conjunction with the immunosuppressant dosing and for two weeks following cessation of immunosuppressants.

αGLA Immune Response

Blood samples will be drawn to assess the immune responses directed against αGLA; the transgene expressed in the FLT190 therapy. The development of neutralizing and non-neutralizing immune responses will be monitored by measuring anti-αGLA antibodies at a central laboratory.

AAVS3 Immune Response

Blood samples will be drawn for the assessment of the innate and adaptive immune responses directed against AAVS3; the capsid used in the FLT190 therapy. The cell mediated immune response and development of anti-AAVS3 antibodies will be analyzed at a central laboratory.

Vital Signs

Measurements of vital signs (blood pressure, pulse, temperature, and respiratory rate) will be performed according to the study schedule. Blood pressure and pulse will be determined in the sitting position (for 5 minutes).

Blood pressure should be determined by cuff (manual or automated is acceptable although the same method should be used throughout the study).

Any clinically significant deviations from vital signs at Day -1 should be reported as an AE.

Electrocardiogram

A 12-lead ECG will be conducted and analyzed locally at the study sites. Any clinically significant deviations from Day -1 should be reported as an AE.

24-hour Holter Monitor

A 24-hour Holter monitor will be conducted in accordance with the study schedule. Any clinically significant abnormalities (e.g. high ectopy burden and runs of arrhythmia) from screening should be reported as an AE.

Echocardiogram

An echocardiogram will be conducted in accordance with the study schedule. Any clinically significant abnormalities (e.g. change in ejection fraction, regional wall motion abnormalities) from screening should be reported as an AE.

Liver Ultrasound

A liver ultrasound will be conducted; any clinically significant deviations from screening should be reported as an AE.

Adverse Event Collection

Subjects will be questioned in a general way at each study visit to establish whether AEs have occurred since the previous visit (e.g., "How have you been feeling since your last visit?").

Additionally, the investigator will evaluate other collected data (e.g., subject diaries, questionnaires, clinical evaluations) to ascertain whether and AE has occurred. AEs are collected from the time initial informed consent is signed until Week 38 (Section 15.2.1).

13.4.4 Vector Shedding

Plasma, saliva, urine, stool, and semen samples will be taken for PCR of vg. These collections will occur at each visit until there have been three prior consecutive specimens that were negative at separate visits (at least 1 week apart). The samples will be analyzed at a central laboratory. Semen samples may be omitted at the discretion of the PI for religious or other personal reasons expressed by the subject.

13.4.5 Health Economic Assessments

Quality of Life

QoL will be evaluated using the EQ-5D-5L questionnaire.

Health Resource Utilization

The following items will be recorded:

- 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 subjects and caregivers (e.g., for ERT dosing).

13.4.6 Exploratory

Estimated Glomerular Filtration Rate

eGFR will be derived using the combined cystatin C and serum creatinine CKD-EPI equation. Creatinine and cystatin C levels will be analyzed by the central safety laboratory as part of the chemistry laboratory safety assessments.

Pain, GI Symptoms, and Fatigue Assessment

Subjects will answer questionnaires relating to pain (SFBPI), GI symptoms (GSRS), and fatigue (FACIT-Fatigue) using an electronic diary.

Subjects will complete the SFBPI questionnaire once a day during the screening period and Day -1, and then for 7 consecutive days before specified visits.

The FACIT fatigue and GSRS questionnaires will be completed by subjects at the end of each week during the screening period and Day -1, and then at specified timepoints during the study.

Magnetic Resonance Imaging (Cardiac and Brain)

A cardiac and brain MRI will be performed. The cardiac MRI will be performed with weight-adjusted gadolinium-based contrast agent. The duration of each MRI is approximately 45 to 60

minutes. MRI will be conducted as outlined in the study imaging manual (including the full list of brain and cardiac parameters to be measured). Images will be analyzed by a central imaging vendor.

Mononuclear Cells (Research)

Blood samples will be collected and mononuclear cells isolated for research purposes. Research samples will be retained beyond the end of the study and may be analyzed alongside samples taken as part of the long-term follow-up study. The samples may be held up until the completion of the long-term follow-up study and analyzed as part of future research which will be ethically approved.

The intent of the research is to evaluate the potential over time for AAV insertions into the host genome. The samples may be held until the completion of the long-term follow-up study and analyzed as part of future research which will be ethically approved. The samples will be analyzed at a central laboratory. The cell samples may also be used to analyze leukocyte αGLA activity levels during the study.

Additional, unscheduled mononuclear cells (research) samples can be collected in cases of transaminitis as described in Section 11.4.2. These additional samples will only be collected at study sites during scheduled study visits.

The samples will be analyzed at a central laboratory.

Biomarker and Research Plasma and Serum

The intent of drawing these samples is to investigate any potential biomarkers that may be involved in the immune response as well as to have samples available for future research purposes. Additional, unscheduled biomarker and research plasma samples can be collected in cases of transaminitis as described in Section 11.4.2.

Each plasma sample collected will be split into two aliquots according to the schedule of assessments.

The first aliquot will be analyzed for immune response biomarkers at the central laboratory.

The second aliquot will be for research purposes. Aliquots intended for research purposes will be retained beyond the end of the study and may be analyzed alongside samples taken as part of the long-term follow-up study. The samples may be held up until the completion of the long-term follow-up study and analyzed as part of future research which will be ethically approved.

Due to the relatively small number of subjects in the study, the likelihood that LFT elevation will only be observed in a proportion of the group and the limitations of these types of analyses, the analysis will be conducted independent of the study report and potentially pooled with other studies using similar constructs to generate a larger sample for analysis. For this reason, these plasma samples may be held until the completion of the long-term follow-up study and analyzed as part of future research which will be ethically approved.

All samples will be analyzed at a central laboratory.

Research Urine

Urine samples will be collected for research purposes. Research samples will be retained beyond the end of the study and may be analyzed alongside samples taken as part of the long-term follow up study. The samples may be held up until the completion of the long-term follow-

up study and analyzed as part of future research which will be ethically approved. The samples will be analyzed at a central laboratory.

Urine Protein and Albumin

Subjects will collect separate urine samples (first morning void/mid-stream) on the day of the site visit and the two days preceding the site visit for spot urine analysis of urine protein and albumin excretion.

The samples will be analyzed at a central laboratory.

13.5 Volume of Blood to be Drawn from Each Subject

During this study, it is expected that approximately 1800 mL of blood will be drawn from each subject for central laboratory assessments.

The protocol allows for monitoring of certain parameters through local laboratories. Blood volume requirements are likely to vary between study sites; however, it is estimated that approximately an additional 400 mL will be drawn from each subject for local laboratory assessments over the course of the study.

Note: The amount of blood to be drawn for each assessment is an approximation. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment.

13.6 Discontinuation/Withdrawal of Participants

The reason for discontinuation/withdrawal must be determined by the investigator and recorded in the subject's medical record and in the eCRF. If a subject is withdrawn for more than one reason, each reason should be documented in the source document and the most clinically relevant reason should be entered in the eCRF.

Reasons for discontinuation include but are not limited to:

- Protocol violation
- Withdrawal by subject
- Death
- Lost to follow-up
- Other (If "other" is selected, the investigator must specify the reason on the eCRF)

If a subject expresses their wish to withdraw from the study, the investigator should explain the importance of remaining on study follow-up, or failing this, of allowing routine follow-up data to be used for study purposes.

In the event of early withdrawal/discontinuation, every effort should be made to collect data in line with Week 38/EOS assessments.

13.6.1 Withdrawal of Consent to Data Collection

If a subject explicitly states they do not wish to contribute further data to the study, their decision must be respected and recorded in the eCRF and medical notes.

13.6.2 Loss to Follow-up

If a subject relocates from the area, every effort should be made for the subject to be followed up at another participating study site and for this new site to take over the responsibility for the subject, or for follow-up via the subject's general practitioner.

If a subject is lost to follow-up at a site, every effort should be made to contact the subject's general practitioner to obtain information on the subject's status.

13.7 Definition of End of Trial

The end of the study will be defined as the last visit by the last subject.

14 Dose Progression, Dose Escalation, and Temporary Halt Rules

The Sponsor will retain final responsibility for decision making on all aspects of dose progression, dose escalation, temporary halt, and stopping rules. Progression of dosing within a cohort, dose escalation/reduction, communication between active sites and the Sponsor, and temporary halt and stopping rules are detailed below and in study specific plans. The following elements will be included.

14.1 Oversight Committees

14.1.1 Independent Data Monitoring Committee

The role of the DMC is to provide independent advice on data and safety aspects of the study. The DMC will review subject data prior to a dose escalation decision (and when requested for cohort expansion) or in the case of arising safety concerns. The DMC will be governed by a charter that dictates constitution and decision making on dose escalation and study temporary halt and stopping rules.

14.2 Dose-Limiting Toxicity

A dose-limiting toxicity (DLT) is defined as any severe AE at least possibly related to FLT190 except for increases in ALT or AST that are not associated with increases in bilirubin. As described in Section 11.4.2, transaminase increases should be investigated to exclude other causes but it is expected that such transaminase increases will be cases of vector-associated transaminitis which is an event anticipated with liver directed AAV gene therapy (Section 7.5.2). In the case of vector- associated transaminitis, where ALT or AST levels are classed as severe, changes to the prophylactic immunosuppressive regimen will be considered for ongoing subjects and before treating further subjects.

The study may be stopped before completion at any time on the recommendation of the DMC or decision by the Sponsor.

14.3 Dose Escalation

αGLA expression kinetics are expected to follow a pattern similar to that observed with other ss AAV gene therapies, with initial response appearing within a few days and rising to approximately 50% of peak levels over the first 2 weeks post-infusion. Following this, trajectory is expected to slow, reaching steady-state levels at approximately Week 12. Based on these expectations the dose intervals between subjects and related rules have been determined and summarized in Section 14.3.1.

14.3.1 Dose Cohorts and Dose Interval Between Subjects

FLT190 will be given as a single dose, slow IV infusion.

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

- Cohort 1: 0.75×10^{12} vg/kg of body weight
- Cohort 2: 1.5×10^{12} vg/kg

- Cohort 3: 4.5×10^{12} vg/kg
- Cohort 4: 1.3×10^{13} vg/kg

Subjects in the USA will only be enrolled for Cohort 2 and above.

Based on the observed safety and αGLA expression within a dosing cohort, an intermediary dose level 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.

Subjects will be treated in a staggered approach.

- Safety and αGLA assessments for dose escalation and cohort expansion rules will take place at least 8 weeks post dosing for subjects in Cohort 1 and 2.
- Safety and αGLA assessments will take place at least 8 weeks post dosing for the first (sentinel) subject in Cohort 3 and 4.
- Safety and αGLA assessments will take place at least 4 weeks post dosing for the second and subsequent subjects in Cohort 3 and 4.
- A cohort may be expanded to include additional subjects, to further inform the safety and efficacy of any given dose level. Dosing in those additional subjects will be staggered by at least 4 weeks post FLT190 administration.
- An interim analysis is planned 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 is to inform the recommended dose for a Phase 3 study in PTPs. When the Part 1 dose escalation is complete (and all patients have at least 12 weeks of data following FLT190 administration), dosing in Part 2 will also be discussed with the DMC to inform the recommended dose for Part 2 in PUPs.

Intervals between dosing subjects will be enforced to monitor for acutely occurring dose-limiting AEs. On review of data following dosing with FLT190, if a subject reaches αGLA expression >2400 nmol/h/mL, no further subjects will be dosed at that or a higher dose. αGLA expression >2400 nmol/h/mL indicates beyond known tolerated αGLA expression.

The DMC will be consulted to reduce dose before dosing the next subject.

14.3.2 Cohort Expansion and Dosing Rules

Cohort expansion and dose escalation/reduction will occur based on the rules outlined in Sections 14.3.3 and 14.3.4. In all cases safety will be assessed first followed by evaluation of αGLA expression.

In Cohorts 1 and 2, at least two subjects will be dosed in each cohort. Depending on assessment of safety, tolerability and αGLA expression additional subjects may be treated.

Subjects in the USA will only be enrolled for Cohort 2 and above.

In Cohorts 3 and 4, three subjects will be dosed in each cohort.

The Sponsor may expand a dose cohort (including a dose reduction) after discussion with the DMC to better characterize the safety profile or αGLA expression.

Upon dose escalation, the Sponsor may increase to an intermediary dose (higher than current cohort but lower than the dose level of the next planned cohort) after discussion with the DMC.

14.3.3 Rules Applicable to Evaluation of Safety in Each Dose Cohort

After first subject has been dosed in a cohort:

- If no DLT occurs, follow dosing rules in relation to αGLA expression and dose a second subject in the cohort (Section 14.3.4).
- If a DLT occurs in the first subject: follow dosing rules in relation to αGLA expression and dose a total of three subjects in the cohort (Section 14.3.4).

After two subjects have been dosed in a cohort:

- If no DLT occurs in either subject at the dose level, follow dosing rules in relation to αGLA expression and inter-subject variability (Section 14.3.4)
- If a DLT occurs in either subject, follow dosing rules in relation to αGLA expression and dose a third subject (Section 14.3.4).
- If a DLT arises in both subjects: consult with the DMC to reduce dose before dosing a third subject.

If a third subject has been dosed:

- If no DLT occurs, follow dosing rules in relation to αGLA expression (Section 14.3.4).
- If a DLT occurs in the third subject, with another DLT observed in either the first or second subject, consult with DMC to reduce dose, before dosing the next subject as part of the next cohort.
- If a DLT occurs in the third subject, but no DLTs occurred in first or second subject, follow dosing rules in relation to αGLA expression (Section 14.3.4).

In Part 2, safety assessment will be based on the total number of subjects that experience a DLT at a specific dose level across Parts 1 and 2, in accordance with the rules above.

14.3.4 Rules Applicable to Assessment of αGLA Expression in Each Dose Cohort

αGLA Expression (Plasma αGLA Activity/Concentration)

If the safety assessment does not result in a change to the planned dose escalation or cohort expansion, αGLA expression will be assessed, through plasma αGLA activity and/or concentration to mitigate the risk of dosing additional subjects at potentially sub-therapeutic or beyond known tolerated αGLA expression.

- Subtherapeutic αGLA expression
    - Plasma αGLA expression that is within the normal range is currently considered insufficient to drive optimal cellular uptake. Estimated normal range of αGLA activity is 4 to 22 nmol/h/mL (dependent on laboratory assay).
    - On review of data following dosing with FLT190:
        - In Cohorts 1 and 2, if plasma αGLA expression is less than the ULN in subjects, provided safety assessment permits, the study may advance to the next cohort.
        - In Cohorts 3 and 4, three subjects will be dosed in each cohort.

- Beyond known tolerated αGLA expression
    - αGLA expression >>2400 nmol/h/mL indicates beyond known tolerated αGLA expression.
    - On review of data following dosing with FLT190, if a subject reaches αGLA expression >2400 nmol/h/mL, no further subjects will be dosed at that or a higher dose. The DMC will be consulted to reduce dose before dosing the next subject.

αGLA Expression (Inter-subject Variability)

- In Cohorts 1 and 2, if αGLA expression in the second subject is similar to the first subject in the cohort (αGLA expression with plasma αGLA activity within ± 20% of each other), then the study will advance to the next dose cohort provided the safety and αGLA expression requirements in rules 1 and 2 above are satisfactorily addressed.
- If αGLA expression is not similar but satisfies the requirements of the cohort expansion rules (1 and 2 above), then a third subject will be recruited to the cohort. After this, the study will advance to the next dose if the safety assessment and the αGLA level dose escalation rules are met.

Part 2 Dose Selection

Once all Part 1 subjects have been treated tolerability will be assessed and αGLA expression determined at 12 weeks post FLT190 administration, and a dose will be selected for Part 2 of the study. If an alternative time point would provide a more stable measure of GLA expression or additional GLA measures are required, a protocol amendment will be performed detailing the revised analysis. Part 2: A single cohort of three PUPs will be enrolled at the dose level selected in Part 1.

14.4 Temporary Halt

Once a temporary halt is applied to the study an internal safety review by the DMC will take place. If the Sponsor deems it appropriate to restart the study, this may be done only following approval by the relevant competent authorities, ethics committees, and with any local approvals required.

14.5 Temporary Halt Rules

Further enrolment into the study will be put on hold in the event of any of the following:

- Death of a subject.
- Severe or medically significant AE with life-threatening consequences.
- Severe or medically significant AE of non-life-threatening consequences, hospitalization or prolongation of hospitalization, disabling or limiting self-care in activities of daily living or urgent intervention indicated in a subject related to FLT190.
- Development of malignancy.
- Development of neutralizing anti-αGLA antibody defined as a positive neutralizing antibody result from the central laboratory in conjunction with a drop in GLA expression and rise in Fabry biomarkers (Gb3, lysoGb3).*

*The Investigator should contact the Medical Monitor if there is a suspicion of neutralizing anti-αGLA antibodies. Transient antibodies, defined as a negative αGLA antibody test after a positive result, with no significant drop in plasma αGLA expression and increase in lysoGb3 level, would not trigger the temporary halt rules.

Subjects who have already received the vector infusion will continue to be followed per the protocol. If, following an internal safety review by the independent DMC, the Sponsor deems it appropriate to restart the study; this can only be done following approval by all relevant competent authorities, ECs and along with any local approvals required.

15 Pharmacovigilance – Recording and Reporting of Adverse Events

15.1 Definitions

Term	Definition
Adverse event (AE)	Adverse event means any untoward medical occurrence associated with the use of a drug in humans, regardless of whether considered drug related
Adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies less certainty about causality than adverse reaction, which means any adverse event caused by a drug.
Adverse event of special interest (AESI)	An AESI is an adverse event that is of special interest and is required to be reported to the Sponsor immediately (no more than 24 hours after learning of the event) (Section 15.2.10).
Serious adverse event (SAE)	Serious adverse event or serious suspected adverse reaction. An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be 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 outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. Life-threatening adverse event or life-threatening suspected adverse reaction. An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or Sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. Hospitalization for pre-existing conditions, including elective procedures, does not constitute an SAE.

	Hospitalization for a study related procedure (eg, administration of IV methylprednisolone) does not constitute an SAE.
Unexpected adverse reaction	Unexpected adverse event or unexpected suspected adverse reaction. An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the IB or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the IB referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the IB listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.
Important medical event	Important medical events that may not result in death, be 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 outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. See Section 15.4 for study specific events that should also be considered as important medical events.

15.2 Adverse Events

15.2.1 Adverse Event Collection Timeframe

The time period for reporting AEs will be from the time the initial ICF is signed by a subject until completion of the study. This includes events occurring during the screening phase of the study, regardless of whether FLT190 is administered.

15.2.2 Assessment of Adverse Events

Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually.

Each AE will be assessed for the following criteria:

15.2.3 Severity

The medical assessment of severity will be determined by using the following definitions:

Category	Definition
Mild	The AE does not interfere with the volunteer's daily routine, and does not require intervention; it causes slight discomfort
Moderate	The AE interferes with some aspects of the volunteer's daily routine, or requires intervention, but is not damaging to health; it causes moderate discomfort
Severe	The AE results in alteration, discomfort or disability which is clearly damaging to health

15.2.4 Causality

The investigator must make the assessment of relationship to IMP and NIMP for each AE (Prednisolone [prednisone], methylprednisolone and tacrolimus are considered to be NIMPs in this study [Section 11.3]). The investigator should decide whether there is a reasonable possibility that the event may have been caused by the IMP/NIMP. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related." Otherwise, if there is any valid reason for suspecting a possible cause-and-effect relationship between the IMP/NIMP and the occurrence of the AE, then the AE should be considered "related." The causality assessment must be documented in the source document(s).

15.2.5 Seriousness

Any AE that meets the criteria for seriousness (Section 15.1) will be documented as such within the source document(s). Hospitalization for pre-existing conditions, including elective procedures do not constitute an SAE. Additionally, hospitalizations required to perform protocol-specified procedures/treatments do not constitute an SAE.

15.2.6 Expectedness

Where an AE is determined as "related" and therefore constitutes an adverse reaction an assessment of expectedness will be determined by the Sponsor/CRO using the following definitions:

Category	Definition
Expected	An adverse reaction which is consistent with the information about FLT190 listed in the Reference Safety Information in the FLT190 IB.
Unexpected	An adverse reaction which is not consistent with the information about FLT190 listed in the Reference Safety Information in the FLT190 IB.*

* This includes listed events that are more frequently reported or more severe than previously reported.

15.2.7 Action Taken

The investigator will describe the action taken in response to the AE in the source document(s). Action taken will be transcribed to the eCRF and categorized to one of the following:

- No action
- Concomitant medication administered
- Any non-drug action taken

15.2.8 Follow-up of Adverse Events

All AEs must be followed to conclusion regardless of whether the subject is still participating in the study. Conclusion can be that the event has resolved or until, in the opinion of the investigator, the event has stabilized or been determined to be chronic. The follow-up must be documented in the source document(s).

15.2.9 Documentation and Reporting of Adverse Events

All AEs should be captured on the appropriate AE pages in the eCRF and in source document(s). In addition to untoward AEs, unexpected benefits outside the FLT190 indication should also be captured on the AE eCRF.

15.2.10 Adverse Events of Special Interest

All AESI are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event). AESI for this study include the following:

- An increase of ALT above upper limit of normal on one occasion.
- ≥50% increase of ALT from baseline on one occasion followed by a further increase from the prior level at the next blood test (at same laboratory).
- Increase in ALT associated with a clinically relevant fall in plasma GLA expression.
- An unplanned increase in immune suppression.*
- A positive test result for SARS-CoV-2 (COVID-19) infection.
- An increase in troponin to greater than twice the upper limit of normal (normal hs troponin-T is <14 pg/mL) if screening troponin is in the normal range OR an increase in troponin to greater than 1.5 times the screening level, if troponin is elevated above the normal range at screening.
- A change in 12-lead ECG deemed clinically significant by the PI.
- A change in 24-hour Holter monitor deemed clinically significant by the PI (e.g. high ectopy burden or runs of arrhythmia).
- A change in echocardiography deemed clinically significant by the PI (e.g. change in ejection fraction).
- Myocarditis.

*An increase in immune suppression includes an increase in dose or duration or number of drugs used above the regimens specified in Section 11.4. This includes a longer taper regimen.

To report AESI to the Sponsor, the investigator should record all case details that can be gathered onto the Serious Adverse Event and AESI Reporting Form and submit it to the CRO pharmacovigilance department as per the instruction for reporting SAEs detailed in Section 15.3.1.

15.3 Serious Adverse Events

15.3.1 Serious Adverse Event Reporting Procedures

All initial SAE reports must be reported by the investigator to the Pharmacovigilance (PV) CRO within 24 hours of the first awareness of the event, applicable fax numbers and e-mail addresses can be found on the study -specific Safety Fax/Email Cover Sheet. All follow-up reports must be submitted within 24 hours of additional information becoming available. The investigator must complete the SAE and AESI Reporting Form and transmit the form to the PV CRO (Section 4).

AESI will be reported to the PV CRO via the same process.

15.3.2 Serious Adverse Event Collection Timeframe

The time period for reporting SAEs will be from the time the initial ICF is signed by a subject until completion of the study. This includes events occurring during the screening phase of the study, regardless of whether FLT190 is administered.

In addition, any SAE(s) considered "related" to the FLT190 and discovered by the investigator at any interval after the study has completed must be reported to the Sponsor within 24 hours of the first awareness of the event.

15.3.3 Serious Adverse Event NIMP Causality Assessment

As per Section 15.2.4, the investigator must make the assessment of causal relationship for any SAE to the IMP/NIMP that the subject has received.

15.3.4 Follow-up of Serious Adverse Events

All SAEs must be followed to conclusion regardless of whether the subject is still participating in the study. Conclusion can be that the event has resolved or until, in the opinion of the investigator, the event has stabilized or been determined to be chronic. The follow-up must be documented in the source document(s).

15.3.5 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates. As noted in Section 15.2.5, hospitalizations required to perform protocol-specified procedures/treatments do not constitute an SAE.

In addition, any signs or symptoms experienced by the subject leading up to the onset date (and after initial consent to the study), or following the resolution of the SAE, must be recorded as an AE, if appropriate.

15.3.6 Documentation and Reporting of Serious Adverse Events

As well as the expedited reporting noted in Section 15.3.1, SAEs should be captured on the appropriate AE pages in the eCRF and in source document(s).

15.3.7 Fatal Outcome

Any SAE that results in the subject's death (i.e., the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

15.4 Important Medical Events

The following will be considered as important medical events and will be reported in line with SAE reporting procedures.

- Development of a malignancy.
- Development of a suspected αGLA neutralizing antibody defined as a positive result from the central laboratory in conjunction with a drop in αGLA expression.*
- A subject develops a severe allergic reaction related to administration of FLT190.

*The investigator should contact the Medical Monitor if there is a suspicion of neutralizing anti-αGLA antibodies.

15.4.1 Barrier Protection/Contraception/Pregnancy

The population under study is male; therefore, pregnancies in study subjects are not applicable. Male subjects must practice a reliable barrier method of contraception, until three consecutive negative semen samples taken at separate visits (at least 1 week apart) have been obtained after FLT190 administration. If the subject does not provide semen samples, barrier contraception must be continued until 3 consecutive negative serum samples taken at separate visits (at least 1 week apart) have been obtained after FLT190 administration. In programs to date with our proprietary capsid and in the literature, semen samples become negative before serum samples. Subjects must refrain from sperm donation for the duration of the trial.

Subjects must refrain from sperm donation for the duration of the trial.

If the partner of a male subject becomes pregnant at any time during the post dosing follow up period as part of this study or the long term follow up study, the site team should request for permission to keep in touch with their partner to follow-up the pregnancy to its conclusion as well as the child for the first year of life.

Consent to report information regarding the pregnancy must be obtained from the subject's pregnant partner. A study -specific pregnancy monitoring information sheet and ICF for partners of study subjects must be used for this purpose.

The pregnancy should be reported on a pregnancy reporting form following consent and submitted to the PV CRO (Section 4).

The PV CRO must be kept informed of any new developments involving the pregnancy.

15.4.2 Regulatory Agency, Ethics Committee, and Site Reporting

The Sponsor is responsible for notifying the relevant central regulatory authorities and central ethics committees of related, unexpected SAEs (SUSARs) in accordance with the regulatory requirements. SUSARs that are fatal or life-threatening must be notified with 7 days from the Sponsor learning of them with any additional relevant information reported within a further 8 days. Other SUSARs must be reported within 15 days from the Sponsor learning of them. The Sponsor will also inform all participating sites.

In addition, the Sponsor is responsible for notifying relevant regulatory authorities or the NIMP marketing authorization holder of SAEs that are deemed as related to NIMPs.

The investigator is responsible for notifying the local ethics committee or the relevant local regulatory authority of all SAEs that occur at their site, as required.

The Sponsor will submit an annual Development Safety Update Report to relevant ethics committee and regulatory authorities.

15.4.3 Expedited Reporting of Other Safety Events

New events related to the conduct of the study or the development of the FLT190 which are likely to affect the safety of the subjects should be reported according to the existing timelines for expedited reporting.

This includes:

- SAEs which could be associated with the study procedures and which could require modification of the conduct of the study.
- Events which pose significant hazard to the participant population.
- SAEs related to mandatory concomitant medication, product application process (surgical or other) and product failure (including lack of efficacy) should all be considered.
- Events (as described above) which are fatal or life-threatening, will be notified to the ethics committee and competent authority by the Sponsor/CRO within 7 days of the Sponsor/CRO learning of them. Events falling into one of the other categories of serious (Section 15.1) will be reported by the Sponsor/CRO to the ethics committee and competent authority within 15 days after the Sponsor/CRO has learned of them.

Expedited reporting and reporting of AEs will be in accordance with EudraLex Volume 10, Chapter II (Detailed Guidance on the collection, verification and presentation of AE/reaction reports arising from clinical trials on medicinal products for human use (‘CT-3’; 2011/C 172/01), June 2011) in the European Union (EU) and with 21 Code of Federal Regulation Part 312 to the Food and Drug Administration.

Expedited reporting and reporting of AEs to the Food and Drug Administration will be in accordance with 21 Code of Federal Regulation Part 312.

Should an event also be considered as urgent safety measures, it must be reported in line with Section 15.5.

15.5 Urgent Safety Measures

Urgent safety measures can be put in place with immediate effect without needing to gain prior authorization by the ethics committee (and competent authority where applicable), to protect clinical study subjects from any immediate hazard to their health and safety.

Implementation of urgent safety measures should be notified immediately to the Sponsor who will be responsible for immediate (and no later than 3 days from the date of the measure) onward written notice to relevant regulatory authorities and ethics committees including details of the measure(s) taken and the circumstances giving rise to those measure(s).

16 Statistics and Data Analysis

16.1 Sample Size Calculation/Determination of Sample Size

The number of subjects enrolled in the study will depend on the number of DLTs and αGLA expression observed in each cohort and the number of dose levels evaluated. Overall, the study will include a minimum of 13 and a maximum of 15 subjects (up to 12 subjects enrolled into Part 1 and an additional three subjects enrolled into Part 2). Across Parts 1 and 2, up to six subjects may be enrolled at a single dose level in four different cohorts.

Because of the limited number of subjects in the Fabry population, the sample size of this study is based on pragmatic rather than statistical considerations.

The dose escalation rules aim to minimize the number of subjects that would need to be dosed at suboptimal levels whilst allowing evaluation of safety, with the option to expand a group on observation of dose-limiting AEs.

16.2 Statistical Analysis Plan

All study data will be listed and all relevant data will be tabulated and summarized by dose level, patient population, and overall, where applicable. A Statistical Analysis Plan (SAP) will be written and finalized prior to database lock. This plan will give a detailed description of all summaries and analyses that will be presented.

Statistical analysis will be performed using SAS® (SAS Institute Inc., Cary, NC, USA) statistical software (Release 9.4 or later).

Continuous variables will be summarized using number of observations, mean and standard deviation, median, minimum, and maximum values.

Categorical values will be summarized using number of observations and percentages.

16.3 Procedures for Reporting Any Deviation(s) From the Original Statistical Plan

Any deviations from the analyses planned in the protocol will be detailed in the SAP, and deviations from the planned analyses will be captured in the clinical study report.

16.4 Demography and Baseline Characteristics

Baseline parameters and demography (e.g. age, gender, ethnicity) will be summarized.

Fabry Disease history, ERT/PCT history, and medical history will be summarized using number of observations and percentages of subjects reporting each category.

Exposure to investigational product (i.e., total amount of study drug received) will be listed for all subjects by cohort.

16.5 Study Populations

16.5.1 Analysis Population

The Full Analysis Set will include all subjects who received FLT190. This will be the primary population for all analyses.

16.6 Primary Endpoint

16.6.1 Safety

AEs will be assessed as mild, moderate, or severe, as defined in Section 15.2.3. Frequency of treatment-emergent AEs and SAEs will be calculated for each body system and preferred term, and by dose level, for number of events and number of subjects reporting the event. The severity of the AEs and the relationship to study medication will be summarized for each body system and preferred term by dose level. Frequency of DLTs, defined as any severe AE at least possibly related to FLT190 except for increases in ALT or AST that are not associated with increases in bilirubin, will be summarized.

16.7 Secondary Endpoints

16.7.1 Safety

Descriptive statistics will be calculated for clinical laboratory tests at applicable visits.

Changes from baseline will also be presented. For laboratory data, abnormal values will be flagged in the data listings.

Vital signs (systolic and diastolic blood pressure, temperature, and pulse), ECGs, 24-hour Holter monitor, cardiac MRI, liver ultrasound, and physical examination will be summarized by dose level using appropriate descriptive statistics.

Withdrawals from the study will be summarized by cohort.

16.7.2 Efficacy

Plasma and Urine Gb3 and LysoGb3

Change from baseline in Gb3 and lysoGb3 will be summarized in tabular and graphical format for each subject and overall.

Renal and Skin Biopsy

Volume fraction of Gb3 inclusions per cell will be summarized by renal/skin cell types.

16.7.3 Immune Response Against αGLA

Immune response to the αGLA transgene product will be assessed by measurement of the level of antibodies directed against αGLA. antibody levels will be summarized and plotted.

16.7.4 Shedding

Clearance of vg in blood (plasma), saliva, urine, stool, and semen will be summarized.

16.7.5 Pharmacokinetic

Change from baseline in plasma αGLA activity and concentration will be summarized in tabular and graphical format for each patient and overall.

Overall αGLA activity and concentration (AUC) will be calculated from baseline to Week 38 and summarized.

16.8 Exploratory Endpoints

16.8.1 Efficacy

Leukocyte αGLA Activity Levels

Change from baseline in leukocyte αGLA activity levels up to Week 38 will be summarized.

eGFR

Change from baseline in eGFR up until 38 weeks after treatment will be summarized.

Urine Protein and Albumin Excretion

Change from baseline in urine protein and albumin excretion up until 38 weeks after treatment will be summarized.

Cardiac and Brain MRI

Change from baseline in cardiac parameters and brain imaging measured using MRI will be summarized. The full list of parameters to be measured will be listed in the study imaging manual.

MSSI and FASTEX

Change from baseline in Fabry disease symptom scores, using the MSSI and FASTEX will be summarized.

Note, as FASTEX is being introduced as part of a protocol amendment, for some subjects there may not be a baseline FASTEX value. These subjects may be excluded from any change from baseline descriptions if a baseline FASTEX value is not available.

Requirement for commencement or resumption of Fabry treatment

The occurrence and timing of any commencement/recommencement of ERT or PCT will be summarized.

Symptomatic Pain

Change from baseline in symptomatic pain assessed up until 38 weeks after treatment will be summarized using the SFBPI.

GI Symptoms

Change from baseline in GI symptoms assessed up until 38 weeks after treatment will be summarized using the GSRS.

Fatigue Symptoms

Change from baseline in fatigue assessed up until 38 weeks after treatment will be summarized using the FACIT-Fatigue questionnaire.

Plasma Immune Biomarker Response

Change from baseline in blood plasma immune biomarkers will be summarized.

16.8.2 Immune Response Against AAVS3

Immune response against the AAVS3 capsid will be assessed by the following and summarized:

- Measurement of antibodies directed against AAVS3 capsid at baseline and after dosing
- Measuring the cell mediated response against the AAVS3 capsid in peripheral blood mononuclear cell at baseline and after dosing.

16.8.3 Quality of Life and Health Resource Utilization

Quality of Life

Change from baseline in QoL up until 38 weeks after treatment evaluated using the EQ-5D-5L questionnaires will be summarized.

Health Resource Utilization

Change from baseline in the level of health resource utilized by subjects will be summarized overtime, 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 subjects and caregivers.

16.9 Subgroup Analysis

No subgroup analysis will be performed for this dose-escalation study.

A DMC will review the safety results from a given cohort before escalating to the next dose in a new cohort.

16.10 Handling of Missing Data

Missing data will not be replaced. Analyses will be performed considering all data observed for the respective analysis sets. For missing AEs and concomitant medication start and end dates the followings rules will be applied:

Partial/missing start date:

- Missing day: impute the first of the month unless month is same as month of first dose of study drug, then impute first dose date.

- Missing day and month: impute 1st January unless year is the same as first dose date, then impute first dose date.
- Partial/missing end date:
- Missing day: impute the last day of the month unless month is same as month of EOS visit, then impute day prior to EOS visit.
- Missing day and month: impute 31st December unless year is the same as EOS visit, then impute day prior to EOS visit.

16.11 Protocol Deviations

Protocol deviations will be addressed at monitoring on an ongoing basis. All protocol deviations are to be recorded with the indication of whether they are major as determined by the study management team, in cooperation with data management, medical monitoring, and the Sponsor. These data will be imported into SAS and listed only, including the assignment of minor or major. A review of the protocol deviations will be performed before database lock.

16.12 Interim Analysis

An interim analysis is planned 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 is to inform the recommended dose for a Phase 3 study in PTPs. When the Part 1 dose escalation is complete (and all patients have at least 12 weeks of data following FLT190 administration), dosing in Part 2 will also be discussed with the DMC to inform the recommended dose for Part 2 in PUPs. Additional interim analyses may be added. Planned interim analyses will be detailed in the SAP.

17 Data Management

17.1 Data Collection Tools and Source Document Identification

17.1.1 Source Data

ICH E6 (R2) Section 1.51, defines source data as 'All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).'

The basic concept of source data is that it permits not only reporting and analysis but also verification at various steps in the process for the purposes of confirmation, quality control, audit, or inspection. A number of attributes are considered of universal importance to source data and the records that hold those data. These include that the data and records are accurate, legible, contemporaneous, original, attributable, complete, consistent, enduring, and available when needed.

17.1.2 Source Documents

ICH E6 (R2) Section 1.52, defines source documents as 'Original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial).'

The investigator is responsible for maintaining accurate medical records (source documents) from which information is recorded in eCRFs.

A source document identification list will be implemented prior to the start of the study to identify which data is recorded firstly into source documents, such as medical notes, and then transcribed into the eCRF.

The eCRF will not be utilized as source for any data in this study.

17.1.3 Electronic Case Report Form

Data for each subject will be recorded in a study-specific eCRF by sites.

Source data contained in source documents must be accurately transcribed into the eCRF.

All eCRFs must be completed by staff that are listed on the site staff delegation log and authorized by the Investigator to perform data collection and handling. It is the responsibility of the Investigator to ensure the accuracy of all data entered in the eCRF. All data sent to the Sponsor will be endorsed by the Investigator.

The eCRF will not be utilized as source for any data in this study.

17.1.4 Data Handling and Analysis

A study-specific data management plan will be in place for the study. This will contain details of the software to be used for the database, the process of database design, coding, data entry, data quality checks, data queries, data security (including data base access), database lock and data transfers.

18 Monitoring, Audit and Inspection

18.1 Monitoring

The Sponsor will determine the appropriate level and nature of monitoring required for the study. Risk will be assessed on an ongoing basis and adjustments made accordingly.

The degree of on-site monitoring (if required) will be proportionate to the objective, purpose, phase, design, size, complexity, blinding, endpoints, and risks associated with the study.

A study-specific monitoring plan will be in place for the study. The study will be monitored in accordance with the agreed monitoring plan which may include elements of remote monitoring in cases where appropriate.

The investigator must permit authorized representatives (study monitors) of the Sponsor access to the site to inspect the facilities, essential documents, study data including source data. The study monitor (and auditors, ethics committees, or regulatory inspectors) may check the eCRF entries against the source documents.

18.2 Audit and Inspection

The Sponsor and investigator must permit authorized representatives of the respective national, local, or foreign regulatory authorities, ethics committees, auditors, and/or Sponsor to inspect facilities and to have direct access to essential documents, study data and original source records relevant to this study, regardless of media.

19 Ethics and Regulatory Consideration

19.1 Good Clinical Practice Compliance

19.1.1 Sponsor Responsibilities

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, ICH GCP Guideline E6 (R2) (2016), EU Directive 2001/20/EC, as well as all applicable national and local laws and regulations.

19.1.2 Investigator Responsibilities

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (R2) (2016), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

19.2 Ethics Committees

It is the responsibility of the investigator to ensure that all aspects of ethical review are conducted in accordance with regulatory and local institution requirements.

Before initiation of the study at each study center, the protocol, the ICF, other written material given to the subject, and any other relevant study documentation will be submitted to the appropriate ethics committee. The Sponsor must receive a letter documenting ethics committee approval, which specifically identifies the protocol, protocol number, and ICF, prior to the initiation of the study and release of FLT190 for shipment to site. Any necessary extensions or renewals of ethics committee approval must be obtained for changes to the study such as amendment to the protocol, the ICF, or other study documentation. The written approval of the ethics committee together with the approved protocol and ICF must be filed in the study files.

A progress report must be submitted to the ethics committee at required intervals and at least annually.

At the completion or termination of the study, the investigational site must submit a close-out letter to the ethics committee and the Sponsor.

19.3 Regulatory Authorities

Relevant study documentation will be submitted to the regulatory authorities of the participating countries according to local/national requirements for review and approval before the initiation of the study. On completion of the study, the regulatory authorities will be notified that the study has ended.

19.4 Ethical Conduct of the Trial

The investigator(s) and all parties involved in this study should conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, ICH GCP guidelines, and the applicable national and local laws and regulatory requirements.

It is the investigator's responsibility to ensure that adequate time and appropriately trained personnel are available at the site prior to commitment to participate in this study. The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study -related tasks and shall, upon request of the Sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study Sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform the primary care physician of the subject's participation in the study.

19.5 Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at a site, the investigator will promptly inform the Sponsor and the ethics committee and provide them with a detailed written explanation. The investigator will also return all study materials to the Sponsor. Upon study completion, the investigator will provide the Sponsor, ethics committee, and regulatory agencies with final reports and summaries as required by (inter)national regulations.

Communication with local ethics committee to ensure accurate and timely information is provided at all phases during the study and may be done by the Sponsor, applicable contract research organization, investigator, or, for multicenter studies, the coordinating Investigator according to national provisions and will be documented in the investigator agreement.

19.6 Informed Consent

The process of obtaining informed consent must be in accordance with applicable regulatory requirement(s) and must adhere to GCP.

It is the responsibility of the investigator to ensure that appropriate informed consent has been obtained from all study subjects prior to any study -related procedures. It should be emphasized that the subject may refuse to enter the study or may choose to withdraw from the study at any time, without consequences for their further care or penalty or loss of benefits to which the subjects are otherwise entitled. Subjects who refuse to give or who withdraw written informed consent should not be included or continue in the study.

The investigator or designated personnel will inform the subject of the objectives, methods, anticipated benefits, and potential risks and inconveniences of the study. The subject should be given every opportunity to ask for clarification of any points they do not understand and, if necessary, ask for more information. At the end of the interview, the subject will be given ample time to consider the study. Subjects will be required to sign and date the ICF.

The Investigator will provide the Sponsor with a copy of the ICF, where applicable, which was reviewed by the ethics committee and which received their favorable opinion/approval. A copy of the ethics committee's written favorable opinion/approval of these documents must be provided to the Sponsor before the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party

(i.e., Sponsor or coordinating investigator) is responsible for this action. Additionally, if the ethics committee requires modification of the sample subject information and consent document provided by the Sponsor, the documentation supporting this requirement must be provided to the Sponsor.

As per Section 13.1.2 study subjects may be rescreened should the screening window elapse. Subjects that are rescreened into the study will receive a new subject number and will be required to reconsent for participation in the study.

Signed consent forms must remain in each subject's study file and must be available for verification at any time. A copy of the signed ICF will be given to the subject. The original signed form will be retained at the study site, and a copy will be placed in the medical notes.

19.7 Notification of Serious Breaches to Good Clinical Practice and/or the Protocol

A "serious breach" is a breach which is likely to effect to a significant degree:

a) the safety or physical or mental integrity of the subjects of the study; or
b) the scientific value of the study.

The sponsor of a clinical study shall notify the licensing authority in writing of any serious breach of:

a) the conditions and principles of GCP in connection with that study; or
b) the protocol relating to that study, as amended from time to time, within 7 days of becoming aware of that breach.

The Sponsor and CRO will be notified immediately of any case where the above definition applies during the study conduct phase.

19.8 Indemnity/Liability and Insurance

The Sponsor of this study adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document will be supplied to the investigator before study initiation, per local country guidelines.

The Sponsor will ensure that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate will be supplied to the investigator as necessary.

19.9 Public Posting of Trial Information

The Sponsor is responsible for posting appropriate study information on applicable websites, as required. Information included in clinical study registries may include participating investigators' names and contact information.

19.10 Protocol Amendments

All proposed protocol amendments will be assessed by the Sponsor and classified as substantial or non-substantial. Substantial amendments will require review and approval by the relevant ethics committee and/or regulatory authority (depending on the nature of the amendment) prior to being implemented, except where required to prevent or ameliorate immediate harm to patients (Section 15.5). Other local or national approvals may be required and will be sought accordingly prior to implementation.

19.11 Trial Suspension, Termination and Completion

The Sponsor may suspend or terminate the study at any time for any reason. If the study is suspended or terminated, the Sponsor will ensure that applicable sites, regulatory agencies, and ethics committees are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The Sponsor will make an end-of-study declaration to the relevant competent authority as required by Article 10(c) of EU Directive 2001/20/EC.

19.12 Submission of Summary Clinical Study Report to Competent Authorities and Ethics Committees

The Sponsor will provide a summary of the clinical study report to the competent authority and, where applicable, the ethics committee of the member state(s) concerned as required by regulatory requirements.

19.13 Record Keeping and Archiving

Essential documents are those documents that individually and collectively permit evaluation of the conduct of the study and quality of the data produced with the principles of GCP and all applicable regulatory requirements. Essential documents relating to the study will be kept in an orderly manner, and remain current, by the Sponsor and investigators in secure study files during the study. At the end of the study, all essential documentation and study data must be archived securely by the Sponsor and study sites according to ICH GCP requirements and Advanced Therapy Regulations (1394/2007/EC) for a minimum of 30 years. These files will be available for inspection by the regulatory authorities or Sponsor and its representatives.

Essential documents and study data must be maintained and may not be destroyed without written permission from the Sponsor. It is the responsibility of the Sponsor to inform study sites when these no longer need to be retained. An investigator must contact the Sponsor before destroying any study -related documentation. In addition, all subject medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

19.14 Privacy and Confidentiality

The Sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number. Where permitted, the subject's year of birth (and age at entry to study) may also be collected and used to assist the Sponsor to verify the accuracy of data (e.g. to confirm laboratory results have been assigned to the correct subject).

Study data containing subjects' unique identifying number and relevant information from their medical records will be recorded and will be available in the results of the study. The data and results may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data

analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

20 Publication Policy

The Sponsor intends to publish the results of this study in accordance with external guidelines regardless of whether the outcomes are perceived as positive, neutral, or negative.

All publications relating to the Sponsor's projects must undergo appropriate technical and intellectual property review and require sponsor agreement to publish prior to release of information. The review is aimed at protecting the Sponsor's proprietary information existing either at the commencement of the study or generated during the study.

If the study is part of a multicenter study, the first publication of the study results shall be made by the Sponsor in the form of a multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the Sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the Sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from their specific site in accordance with this section. The Investigator must, however, acknowledge in the publication the limitations of the single site data being presented.

No publication that incorporates the Sponsor's confidential information shall be submitted for publication without the Sponsor's prior written agreement to publish and shall be given to the Sponsor for review at least 60 days prior to submission for publication. If requested in writing by the Sponsor, the institution and Investigator shall withhold submission of such publication for up to an additional 60 days to allow for filing of a patent application.

The Sponsor must review and approve any results of the study or abstracts for professional meetings prepared by the investigator(s). Published data must not compromise the objectives of the study. Data from individual study centers in multicenter studies must not be published separately.

21 Funding

This study is fully funded by Freeline.

22 Statement of Compliance

The study will be conducted in compliance with the approved protocol, ICH GCP, and applicable regulatory requirements.

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Schiffmann R, Goker-Alpan O, Holida M, et al. Pegunigalsidase alfa, a novel PEGylated enzyme replacement therapy for Fabry disease, provides sustained plasma concentrations and favorable pharmacodynamics: A 1-year Phase 1/2 clinical trial. J Inherit Metab Dis. 2019;1-11.

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Wilcox WR, Linthorst GE, et al. Anti-α-galactosidase A antibody response to agalsidase beta treatment: data from the Fabry Registry. Mol Genet Metab. 2012;105(3):443-449.

Tsukimura T, Nakan S, et al. Plasma mutant α-galactosidase A protein and globotriaosylsphingosine level in Fabry disease. Molecular Genetics and Metabolism Reports. 2014;1:288–298.

24 Appendices

24.1 Appendix 1: Treatment Management Plans

Management of Anaphylaxis

Patients will be informed in advance of potential early symptoms and signs of hypersensitivity reactions, including hives, generalized urticaria, angioedema, chest tightness, dyspnea, wheezing, faintness, hypotension, tachycardia, and anaphylaxis. Patients will remain under observation in the investigational center for at least 8 hours following infusion to minimize the risk associated with acute allergic reaction. Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored at 15-minute intervals from the start of infusion. Vital signs will be monitored hourly from the end of the infusion i.e. +1, +2, 3, 4, 5 and 6 hours. Further measurements will be taken at +8 hours and every 2 hours if deemed necessary by the investigator.

Should anaphylaxis occur, then initial management will consist of standard acute control measures - epinephrine, antihistamine and steroid, followed by intensification of immune suppression by means of continued steroid, cyclosporine A and cyclophosphamide.

Management of Immune Complex Disease

Immune complex disease will be managed by immune suppression with agents detailed in the following review:

Houssiau FA, Lauwerys BR. Current management of lupus nephritis Best Practice & Research. Clinical Rheumatology. 27(2013):319–328.

Management of Infusion Reaction

Whilst acute infusion reaction is deemed highly unlikely in view of its absence in over 50 infusions of varying concentrations and amounts of AAV vector for gene therapy, patients will remain under observation in the investigational center for at least 8 hours following infusion. Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored at 15-minute intervals from the start of infusion. Vital signs will be monitored hourly for 6 hours from the end of the infusion i.e. +1, +2, 3, 4, 5, and 6 hours. Further measurements will be taken at +8 hours and every 2 hours if deemed necessary by the investigator. Should they occur, infusion reactions will be managed in the same way as transfusion associated reaction.

Faed J, 2014, www.nzblood.co.nz; Guidelines for management of adverse transfusion reactions.

24.2 Appendix 2: Renin Angiotensin System Blocker (RASB) Treatment Protocol

RASB concomitant treatment is required for patients with evidence at screening or a medical history of the equivalent of 500 mg/24 hours (PCR in mg/g approximates protein excretion in mg/24h) or more of urinary protein:

Patients currently receiving a RASB

If a patient is already receiving a RASB, if required, the dose will be increased to at least an equivalent of ramipril 10 mg (if on angiotensin converting enzyme [ACE] inhibitor [ACEI]) or irbesartan 300 mg (if on an angiotensin receptor blocker [ARB]), if acceptable to the patient's primary physician, if tolerated and if not contraindicated (see below).

Patients not currently receiving a RASB

Patients who are not already receiving a RASB will be prescribed and instructed to start taking 10 mg of ramipril daily or 300 mg of irbesartan daily (if ramipril is contraindicated or has side effects) or another ACEI or ARB at equivalent doses if there are impediments to the use of ramipril or irbesartan.

Patients who are contraindicated to RASB, or not show no evidence or medical history of proteinuria

Patients who have contraindications to RASB (e.g., SBP<100 mmHg, K+>5.5 mEq) or do not have evidence or history of proteinuria as defined as the equivalent of 500 mg/24 hours (PCR in mg/g approximates protein excretion in mg/24h) are normotensive and are not being treated with RASB or other anti-hypertensive agents will not be treated with these drugs, as this represents the standard of care.

Further instruction

Patients who are receiving RASB for the first time as part of this study will start with half a dose; if there are no side effects, this will be increased to a full dose at after two weeks and their serum K+ and creatinine measured after 2 weeks. RASB will be immediately discontinued in the case of allergic reactions or angioedema. Their dose will be decreased to half if symptomatic hypotension (SBP<100 mmHg) or intractable cough develops, followed by their discontinuation. For persistent cough with ramipril or other ACEI, irbesartan or another ARB will be prescribed in substitution of the ACEI.

In the case of hyperkalemia (K+ >6.0 mEq) or serum creatinine elevation (>30% increase over baseline values), the patient will be asked to immediately obtain a confirmatory laboratory value and then discontinue the RASB while awaiting this confirmatory result. If confirmed, the patient will resume RASB at half dose 72 hours later and will have repeat labs one week later. If the problem persists, RASB will be discontinued for the remainder of the study and BP managed by alternate drugs as needed (see below). If not confirmed, the participant will resume RASB at their usual dose and have a repeat laboratory check one week later.

These same steps will be taken if hyperkalemia develops during the study.

Patients will continue to take any other antihypertensive drug that they may have been taking before study entry. Hypertensive patients will be provided with a blood pressure monitoring device (if they do not already have access to one), will be trained on its use, and will be instructed to periodically monitor their blood pressure at home and to record the results into a

BP diary, and to communicate them to study personnel if values are abnormal. The goal will be to maintain BP <140/90 mm Hg.

If hypotension develops (SBP<100 or significant light-headedness), the dosage of non-RAS antagonist antihypertensive drugs will be progressively reduced until discontinuation, followed by a reduction of RASB to half the dose and their discontinuation if the problem persists. If BP is found to be elevated (>140/90 mm Hg) on three consecutive occasions, the dosage of existing non-RAS antagonists antihypertensive drugs will be maximized, followed, if necessary, by the introduction of antihypertensive drugs of a different class. These will be chosen in collaboration with the other health care providers that are involved in managing the patient's anti-hypertensive therapy. If the goal of BP ≤140/90 is not achieved with these drugs, the possibility of causes of hypertension other than Fabry disease should be considered. BP will continue to be monitored and the anti-hypertensive therapy to be adjusted in a similar way throughout the study.

Letters will be written to the participants' physicians informing them about the study and notifying them of the RASB requirements and blood pressure goals. The letter will propose active participation of the participant's physicians in blood pressure management.

Approximate Dose Conversion for ACE Inhibitors

DRUG	LOW	MEDIUM	HIGH
Benazepril (Lotensin)	5–10 mg/day	20–40 mg/day	60–80 mg**
Captopril (Capoten)	6.25 mg TID	12.5–25 mg TID	37.5*–50 mg TID
Enalapril (Vasotec)	2.5–5 mg/day	10–20 mg/day	30*–40** mg/day
Fosinopril (Monopril)	5 mg/day	10–20 mg/day	30*–40 mg/day
Lisinopril (Prinivil, Zestrin)	2.5–5 mg/day	10–20 mg/day	30–40 mg/day
Moexipril (Univasc) ***	3.75*–7.5 mg/day	11.25*–15 mg/day	22.5–30 mg/day**
Perindopril (Aceon) ***	2–4 mg/day	6*–8 mg/day	12–16 mg/day**
Quinapril (Accupril)	5–10 mg/day	20–40 mg/day	60–80 mg/day**
Ramipril (Atace)***	1.25–2.5 mg/day	5–10 mg/day	15–20 mg/day**

Approximate Dose Conversion for ARB (from GRIPA 2006)

DRUG	LOW	MEDIUM	HIGH
Irbesartan (Avapro)	75 mg	150 mg	300 mg
Olmesartan (Benicar)**	<20 mg (5 mg tabs available)	20 mg	40 mg
Candesartan (Atecand)	4–8 mg	16 mg	32 mg
Eprosartan (Teveten)	400 mg	600–800 mg	
Losartan (Cozaar)	25 mg	50–100 mg	

* Requires pill splitting of ACE inhibitors
** Requires multiple tablets (often less cost-effective)
*** Not available generically

24.3 Appendix 3: Guidance for Investigators on Assessment and Management of Cardiac Events

High sensitivity troponin-T, NT-proBNP levels, ECG, 24-hour Holter monitor, echocardiogram and cardiac MRI will be monitored during the study, in accordance with Table 1. 12-lead ECG and 24-hour Holter monitoring will monitor for arrhythmic abnormalities which may be evident at baseline or on follow-up. Echocardiography will monitor for any structural or functional abnormalities in the heart which may be evident at baseline or on follow-up. Cardiac MRI is performed in screening and locally read for protocol eligibility. Repeat cardiac MRI is performed at the end of study, and centrally read for changes against baseline, in accordance with the study cardiac imaging manual.

Subjects who develop chest pain, hs troponin-T rise, ECG/Holter/echocardiogram changes, or any other signs or symptoms of concern related to a possible cardiac event should be assessed and treated in line with local guidelines, and the investigator must contact the Medical Monitor. Assessment could include serial ECGs, prolonged monitoring with Holter or telemetry, frequent troponin, NT-proBNP, trans-thoracic echocardiogram and a cardiac MRI (with gadolinium) performed as soon as possible, and within 1 week of onset of events. Ideal timeframe for evaluation should be within 72 hours, if possible. Local cardiology review should be considered for management. In the event of ECG changes that the PI feels could be consistent with ischemic heart disease, a coronary angiogram is recommended following cardiology consultation. If this is not possible consideration should be given as to an alternative e.g. CT coronary angiography. The subject is expected to avoid strenuous exertion until after the event has resolved.

24.4 Appendix 4: Management Guidelines for CMV Reactivation

Human cytomegalovirus (CMV) can result in opportunistic infection in patients requiring immunosuppression for their underlying immune disorders such as systemic lupus erythematosus but at a much lower frequency than organ transplantation and hematopoietic stem cell transplantation [Atabani 2012; Lim 2019]. Nevertheless, surveillance for CMV reactivation is necessary in these individuals so that treatment can be instituted early. Natural history studies have demonstrated a correlation between replication kinetics, peak and cumulative viral load with CMV end-organ disease [Atabani 2012]. Systematic review and meta-analysis have also demonstrated the validity of viral load, as determined by real-time PCR (qPCR), as an appropriate surrogate endpoint for predicting the development of CMV disease and guiding pre-emptive therapeutic intervention for the prevention of CMV disease [Griffiths 2016; Natori 2018].

A CMV PCR titer >3000 genomes/mL (or locally agreed on cut-off) should lead to firstly a re-evaluation of the immunosuppression regimen, regardless of any observed clinical symptoms. In clinical study FLT190-01, this would prompt a taper of corticosteroids. We would maintain treatment with tacrolimus to prevent recurrence of transaminitis. Secondly, treatment is clinically indicated if there are any symptoms suggesting CMV infection [Ljungman 2017]. In which case we suggest one of the following: Valganciclovir 900 mg bd orally, Ganciclovir 5 mg/kg bd iv or Foscarnet 60 mg/kg tds IV. Treatment is continued until 2 negative DNA results are obtained. If a subsequent episode is detected after treatment has been stopped, treatment will be reinitiated only if viral load is greater than 3000 genomes/mL.

Atabani, S. F., Smith, C., Atkinson, C., Aldridge, R. W., Rodriguez-Perálvarez, M., Rolando, N., Griffiths, P. D. (2012). Cytomegalovirus replication kinetics in solid organ transplant recipients managed by preemptive therapy. American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons, 12(9), 2457-2464. doi:10.1111/j.1600-6143.2012.04087.x

Griffiths, P. D., Rothwell, E., Raza, M., Wilmore, S., Doyle, T., Harber, M., Emery, V. C. (2016). Randomized Controlled Trials to Define Viral Load Thresholds for Cytomegalovirus Pre-Emptive Therapy. PLoS One, 11(9), e0163722. doi:10.1371/journal.pone.0163722

Lim, C. C., Tan, B. H., Tung, Y. T., Huang, H., Hao, Y., Mok, I. Y. J., Choo, J. C. J. (2019). Risk-stratified approach to anti-viral prophylaxis against cytomegalovirus disease in glomerulonephritis and renal vasculitis treated with potent immunosuppressants. Infectious diseases (London, England), 51(10), 745-752. doi:10.1080/23744235.2019.1648855

Ljungman, P., Boeckh, M., Hirsch, H. H., Josephson, F., Lundgren, J., Nichols, G., Disease Definitions Working Group of the Cytomegalovirus Drug Development, F. (2017). Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials. Clin Infect Dis, 64(1), 87-91. doi:10.1093/cid/ciw668

Natori, Y., Alghamdi, A., Tazari, M., Miller, V., Husain, S., Komatsu, T., Forum, C. M. V. C. (2018). Use of Viral Load as a Surrogate Marker in Clinical Studies of Cytomegalovirus in Solid Organ Transplantation: A Systematic Review and Meta-analysis. Clin Infect Dis, 66(4), 617-631. doi:10.1093/cid/cix793

24.5 Appendix 5: Management Guidelines for Tacrolimus Dosing

Introduction

Tacrolimus is a macrolide immunosuppressant produced by Streptomyces tsukubensis and is classified as a calcineurin inhibitor (CNI). Tacrolimus is an active ingredient in different oral formulations and is available as immediate release (IR) capsules (Prograf) or as granules for suspension (Modigraf) or as prolonged release (PR) capsules (Advagraf). Alternative formulations available in Europe include generic IR capsules Adoport and Tacni, or generic PR capsules, Tacforius. In addition, PR formulations are also available as Tablets (Envarsus). Prograf and Advagraf are used in >90% of patients in Europe for both de-novo and maintenance therapy.

Patients should be maintained on a single formulation of tacrolimus with the corresponding dosing regimen (i.e. prescribing by brand), using a formulation that is commercially available in the site's country. Inadvertent, unintentional, or unsupervised switching of formulations is associated with clinically relevant differences in systemic exposure to tacrolimus, and can lead to increased incidence of side effects, including under- or over immunosuppression. The prolonged release formulations are preferred if available locally.

Target Tacrolimus Whole Blood Trough Level

Target trough levels (equivalent AUC of 200 ng.h/mL) are initially approximately 10-15 ng/ml. During tapering of tacrolimus, target trough level should be maintained between 5-10 ng/mL for two weeks and then 1-5 ng/mL for a further two weeks before stopping.

Timing of Trough Level for Tacrolimus Dose Modifications

For tacrolimus dose modifications, tacrolimus blood concentrations samples should be determined at the end of dosing interval as follows:

- Tacrolimus IR (administered twice daily, morning and evening): Blood samples should be taken at 12 hours in the morning after the previous days evening dose.
- Tacrolimus PR (administered once daily in the morning): Blood samples should be taken at 24 hours after previous days dose.

For both formulations, blood samples should be taken as close as possible to 12 and 24 hours after dosing for IR and PR respectively. A maximum time window of +/- 1 hour is acceptable.

Patients attending the clinic for collection of trough blood samples, MUST be advised NOT to take their morning dose tacrolimus prior to attending the clinic. Patients should bring their tacrolimus medication with them and should take their morning dose AFTER blood sample collection.

Tacrolimus Oral Dose

Initiation of Tacrolimus

Tacrolimus therapy should be initiated at a dose of 0.2 mg/kg/day given orally:

- IR: administered in two divided doses, morning and evening, 12 hours apart
- PR: administered once daily administration at 24-hour intervals.

Note: PR must be taken in the morning, except in subjects who work night shift.

Daily doses should be rounded to the nearest 0.5 mg. In case of IR, if the total daily dose is not divisible into two equal doses, then the higher dose should be administered in the morning (e.g. patient requiring 16.5 mg, IR 8.5 mg should be administered in the morning and IR 8.0 mg in the evening). This is to account for a lower and more variable oral bioavailability of the evening dose (diurnal variation).

The capsules should be swallowed with fluid, preferably water on an empty stomach or at least one hour before or two to three hours after a meal. Grapefruit juice or mixed fruit juice should not be taken with tacrolimus because it is an enzyme inhibitor that can interfere with the metabolism of tacrolimus.

The oral bioavailability of tacrolimus is reduced when oral tacrolimus is administered after a meal. Both the rate and extent of absorption of tacrolimus were reduced when administered with food. The extent of decrease is higher when tacrolimus is administered with high fat diet. Because of this tacrolimus should be administered on an empty stomach or at least one hour before or two to three hours after a meal.

Concomitant use of medicinal products or herbal remedies known to inhibit or induce CYP3A4 may affect the metabolism of tacrolimus and thereby increase or decrease tacrolimus blood levels.

Tacrolimus is a known CYP3A4 inhibitor; therefore, concomitant use of tacrolimus with medicinal products known to be metabolized by CYP3A4 may affect the metabolism of such medicinal products.

Dose Adjustments

The investigator should adjust the patient's dose and modify the tacrolimus dose regimen to maintain tacrolimus trough levels targeted to 15 ng/mL (recommended range 10 to 15 ng/mL). Blood levels should be monitored frequently, at every blood draw, with the frequency of monitoring reduced to weekly once the desired exposure has been reached and once other, potentially interacting treatments, are being used at stable doses. Due to the long half-life of tacrolimus (approximately 1 day in adults), it is recommended that dose adjustments are limited to twice per week as changes in trough levels will occur slowly, usually stabilizing 48 to 72 hours after the dose adjustment is made. In clinical practice, the first opportunity for dose adjustment occurs on Day 2 to 3 after initiation of therapy. Ranges of expected blood levels in Caucasian subjects initiated at a dose of 0.2 mg/kg/day are as follows:

Table A: Proportion of Patients Achieving Tacrolimus Whole Blood Trough Levels in Target Range of 10 – 15 ng/mL (Expected in Caucasian Patients).

Formulation	Day after start of tacrolimus	Approximate number of patients with blood levels (% of patients)					
		< 5 (ng/mL)	≥5-<10 (ng/mL)	≥10-<15 (ng/mL)	>15 - ≤20 (ng/mL)	>20 - ≤30 (ng/mL)	>30 (ng/mL)
IR	2	5	30	25	20	15	5
	3	5	20	25	15	30	5
	4	5	10	40	30	10	5
PR	2	5	35	30	5	15	10
	3	5	35	20	20	10	10
	4	5	20	40	25	15	5

Recommended Dose Adjustments Based on Trough Levels
Dose adjustments (increase or decrease) should be made to maintain tacrolimus level within the recommended target range. The extent of dose changes should be based on observed tacrolimus levels on Days 2 and 3 after start of therapy as described below. Alternative dose adjustments may be made in line with local practice to achieve the target levels.

Corticosteroids (CS): CS are inducers of CYP3A4/5, concomitant administration of CS leads to decrease in tacrolimus blood levels**. Care should be taken when tapering CS which has the potential to increase tacrolimus levels.** Therapeutic drug monitoring should be performed following change in CS dose and tacrolimus dose adjustments made as appropriate.

Table B: Suggested Dose Adjustments Based on Trough Levels Day 2/3 for Target Range 10-15 ng/mL

Observed Blood Levels (ng/mL)	Dose Adjustment
≤5 ng/mL	Increase dose by 50%. Subsequent dose adjustment should be considered after 3 days if necessary, taking into consideration new trough levels
>5 to ≤10 ng/mL	Increase dose by 30%. Subsequent dose adjustment should be considered after 3 days if necessary, taking into consideration new trough levels
>10 to ≤15 ng/mL	No change in dose required
>15 to ≤20 ng/mL	Decrease dose by 10-20%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 15 ng/mL
>20 to ≤30 ng/mL	Decrease dose by 20%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 20 ng/mL
>30 ng/mL	Decrease dose by 30%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 30 ng/mL

Table C: Suggested Dose Adjustments Based on Trough Levels for Target Range 5-10 ng/mL

Observed Blood Levels (ng/mL)	Dose Adjustment
≤5 ng/mL	Increase dose by 30%. Subsequent dose adjustment should be considered after 3 days if necessary, taking into consideration new trough levels
>5 to ≤10 ng/mL	No change in dose required
>10 to ≤15 ng/mL	Decrease dose by 10%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 10 ng/mL
>15 to ≤30 ng/mL	Decrease dose by 20%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 15 ng/mL
>30 ng/mL	Decrease dose by 30%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 30 ng/mL

Table D: Suggested Dose Adjustments Based on Trough Levels for Target Range 1-5 ng/mL

Observed Blood Levels (ng/mL)	Dose Adjustment
≤1 ng/mL	Increase dose by 30%. Subsequent dose adjustment should be considered after 3 days if necessary, taking into consideration new trough levels
>1 to ≤5 ng/mL	No change in dose required
>5 to ≤15 ng/mL	Decrease dose by 10%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 5 ng/mL
>15 to ≤30 ng/mL	Decrease dose by 20%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 15 ng/mL
>30 ng/mL	Decrease dose by 30%. Subsequent dose decrease should be considered after 3 days if tacrolimus trough levels remain above 30 ng/mL

24.6 Appendix 6: Summary of Protocol Changes

Version 7.1 to 8.1

Section(s)	Summary of Change
All	Minor administrative and editorial changes throughout the protocol.
6, 16.1	Addition that 3 PUPs will be enrolled at the dose selected in Part 1. Amendment of sample size.
6	Removal of estimated study completion dates from protocol synopsis.
6, 10.1	Inclusion criterion 5: eGFR amended to $\geq$ 60 mL/min/1.73m^2. Other listed clinical manifestations due to Fabry disease (left ventricular hypertrophy, linear negative slope of eGFR, and neuropathic pain) deleted. Inclusion criterion 6: clarified requirements for urine protein levels at screening. Inclusion criterion 7: re-worded to clarify *willingness to consider* participation in the long-term follow-up study. Inclusion criterion 8: details of barrier contraception replaced with a refence to see the main protocol. Inclusion criterion 10: addition of criterion for Part 2 that subjects must not have received treatment with ERT/PCT. Inclusion criterion 11: addition of inclusion criterion regarding willingness to avoid strenuous exercise during first 3 months after FLT190 administration.
6, 10.2	Exclusion criteria 2: removed (presence of antibodies to αGLA, Replagal, or Fabrazyme). Addition of new exclusion criterion 2: prior hypersensitivity or intolerance to ERT Addition of new exclusion criterion 3: prior lack of response to ERT. Exclusion criterion 3 (new #4): amended CKD stage 4-5 to stage 3-5. Exclusion 4: removed as covered in inclusion criterion 5 (eGFR). Exclusion criterion 5 : myocarditis fibrosis revised to remove 'severe'. Addition of new exclusion criterion 18: addition of history or detection of significant arrhythmia. Exclusion criterion 18 (new #19): revised to include other acute cardiac conditions seemed significant by the investigator. Exclusion criterion 21 (new #22): revised to remove Replagal and Fabrazyme. Exclusion criterion 24 (new #25): revised to include CMV IgG positive subjects who are CMV PCR positive at screening. Exclusion criterion 27: deleted.
6, 7.5.2, 14.3.1	Safety and αGLA assessments for dose escalation and cohort expansion rules revised to place at least 8 weeks post dosing for subjects in Cohort 1 and 2, and for the first subject in Cohort 3 and 4. Safety and GLA assessments in subsequent subjects in Cohort 3 and 4 will occur at least 4 weeks post-dose.
6, 9.1, 14.3.1, 14.3.2	Addition of sentence indicating that subjects in the USA will only be enrolled for Cohort 2 and above.
6, 9.1, 16.12	Addition of an interim analysis to be conducted when dose escalation is complete in PTPs in Part 1 when all patients have at least 12 weeks of data post-dose. This interim analysis will be used to inform the recommended dose for subsequent development.
6, 14.3.2	Addition of option for Sponsor to increase dose to an intermediary dose (higher than current cohort but lower than dose level of next planned cohort) following discussion with DMC.
6, 14.2, 14.5, 15.2.3, 16.6.1	Definition of dose-limiting toxicity revised to be based upon intensity rating of 'severe' instead of CTCAE Grade 3 or higher.

6, 7.5.1, 14.3.1, 14.3.4	Cohort expansion and dose escalations rules reformatted and revised for clarity. Text for subtherapeutic αGLA expression updated to be based upon normal range rather than steady state. Text for αGLA expression beyond known tolerated levels updated to be based upon >2400 nmol/h/mL.
6, 9.1, 14.3.1	Addition of dose cap at a subject weight of 90 kg and addition of body weight dose cap of 90 kg.
6, 9.2.1	Assessment of AEs as primary safety endpoint revised to no longer include CTCAE criteria.
6, 9.2.2, 16.7.1	Secondary safety endpoints revised to include 24-hour Holter monitoring, echocardiogram, cardiac MRI, and physical examination.
6, 9.2.2	Volume fraction of Gb3 inclusions per cell, by renal/skin cell type added as secondary efficacy endpoint.
6, 9.2.2	Anti-AAVS3 antibodies added as exploratory immune response endpoint.
6 (Table 1), 11.4.1, 11.4.2, 13	Addition of: 24-hour Holter monitor (Screening, Week 4, 10, and 38); addition of echocardiogram (Screening, Weeks 4, 8, 12, and 16); troponin (I or T) as local laboratory test (all study visits); hs troponin-T and proBNP as central laboratory tests (all study visits); anti-AAVS3 neutralizing antibody test (Screening); test for tacrolimus level added (Weeks 3-16); CMV test weekly if positive at Screening.
6 (Table 1), 13	Assay names revised: mononuclear cells revised to read AAVS3 immune response (T-cell response). AAVS3 antibody titer revised to AAVS3 immune response (antibodies). αGLA antibodies revised to read αGLA immune response.
6 (Table 1), 13	Clarification of: serum/plasma/urine research samples (Weeks 1-38); Gb3/lysoGb3 (divided into Parts 1 and 2).
6 (Table 1)	Reorganized to group local laboratory tests. Heading row added for central laboratory tests.
6 (Table 1)	Footnotes: Addition of footnote to clarify that Week 1 -32 visits may be conducted at home with a visiting nurse, at Investigator's discretion. Addition of body systems to physical examination. Clarification added to hematology/chemistry labs to include troponin. Addition of footnote that semen samples may be omitted due to religious or other reasons. Renumbered footnotes.
6 (Table 2), 13	Addition of: hs troponin-T at Day -1; central laboratory tests on Day -1 for AAVS3 immune response (antibodies) and αGLA immune response.
6 (Table 2)	Assay names revised as for Table 1.
6 (Table 2)	Footnotes: Addition of body systems to physical examination; timing of collection of urine samples. Footnotes regarding aliquoting of samples deleted as those details will reside in laboratory manual.
7.4	Patient-level data from this study removed from protocol, and cross-reference to current Investigator Brochure added.
7.5.2	Patient-level data from this study removed from protocol, and cross-reference to current Investigator Brochure added. Text on development of neutralizing antibodies reorganized to improve readability/flow.
9.1, 12.3, 13.1.2	Removal of text requiring subjects to complete their latest ERT treatment cycle and then receive at least 2 additional cycles during Screening. Text divided into Part 1 and Part 2 and expanded for clarity. Figure 2 updated.
11.2.5, 11.2.6, 11.2.7	Addition of note that instructions for preparation/handling of infusions are provided in a pharmacy manual.
11.4	Immunosuppressive regimen updated to permit the omission of tacrolimus taper and to give flexibility not to include oral prednisolone in the setting of the reactive regimen. Title of section 11.4.2 updated to reflect reactive regimen.
13.1.1	Text added advising subjects to avoid strenuous exercise for 3 months following vector infusion.

13.2	Addition of text indicating that Week 1 -32 visits may be conducted at home with a visiting nurse, at Investigator's discretion.
13.2.1, 13.4.4	Addition of text indicating that semen samples may be omitted at the discretion of the PI for religious or other personal reasons.
13.4.2	Addition that samples may also be assessed for other related biomarkers e.g. β-galactosidase and hexosaminidase.
13.4.6	Addition of statement defining length of time that aliquots for research purposes may be retained.
13.5	Total volume of blood revised to be approximately up to 1800 mL Removal of sample volumes per test as this information will be in a laboratory manual.
14.5	Addition of temporary halt rules for severe or medically significant AE with life-threatening consequences; and severe or medically significant AE of non-life-threatening consequences, hospitalization or prolongation of hospitalization, disabling or limiting self-care in activities of daily living or urgent intervention indicated in a subject related to FLT190. Clarification of text regarding how development of neutralizing anti-αGLA antibodies will be assessed.
15.1, 15.2.5, 15.3.5	Clarification that hospitalizations for pre-existing conditions, or for a study-related procedure (eg administration of IV methylprednisolone) do not constitute an SAE in and of themselves.
15.2.10	AESI revised to including clinically significant changes in troponin, 12-lead ECG, 24-hour Holter monitor, echocardiography, and myocarditis.
15.4	Development of a malignancy added as an important medical event.
15.4.1	Section renamed to include 'Barrier protection/contraception'. Further detail added regarding length of pregnancy follow-up (up to 1st year of life).
16.2	Update of SAS software to v9.4 or later.
16.6, 16.7, 6.8	Update of analyses in line with changes in parameters described for other sections.
16.10	Removal of 3rd bullet for missing data; the content was addressed in the remaining bullets.
18.2	Deletion of paragraph as regulatory agencies (in general terms) are included in remaining paragraph.
23	Update of reference list.
24.1	Removal of guideline for management of inhibitors, as existing content was not studied in clinical trials in the target population. Investigators will utilize clinical judgment.
24.3	Addition of hs troponin-T, 24-Holter monitor, echocardiogram, and proBNP