FREELINE

ABBREVIATED CLINICAL STUDY REPORT SYNOPSIS

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 (MARVEL 1)

A Multicenter, Long-term, Follow-up Study to Investigate the Safety and Durability of Response Following Dosing of an Adeno-associated Viral Vector (FLT190) in Subjects with Fabry Disease (MARVEL 2)

Protocol Numbers:	FLT190-01 and FLT190-02
Investigational Medicinal Product:	FLT190 (AAV2/S3-FRE1-GLA-co)
Development Phases:	FLT190-01: 1/2 FLT190-02: Long-term Follow-up
Trial Start/Completion Dates:	FLT190-01 First participant (screened): 08 July 2019 FLT190-01 Last visit of last participant: 02 May 2023 FLT190-02 First participant (entered): 08 September 2020 FLT190-02 Last visit of last participant: 24 October 2023
Sponsor:	Freeline Therapeutics Ltd, Sycamore House, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2BP, United Kingdom
Sponsor Signatory:	[redacted]
Coordinating Investigator:	[redacted]
Regulatory Identifiers:	IND number: 024628 EudraCT numbers: 2018-002097-51 (FLT190-01) 2019-004645-32 (FLT190-02) ClinicalTrials.gov numbers: NCT04040049 (FLT190-01) NCT04455230 (FLT190-02)
Report Date:	24 September 2024 (Final v1.0)

ABBREVIATED CLINICAL STUDY REPORT SYNOPSIS

Name of Sponsor/Company: Freeline Therapeutics	**Individual Study Table Referring to Part of the Dossier**	***(For National Authority Use Only)***
Name of Finished Product: FLT190	**Volume:**	
Name of Active Ingredient: FLT190 (AAV2/S3-FRE1-GLA-co)	**Page:**	

Protocol Titles:
FLT190-01: 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 (MARVEL 1)

FLT190-02: A Multicenter, Long-term, Follow-up Study to Investigate the Safety and Durability of Response Following Dosing of an Adeno-associated Viral Vector (FLT190) in Subjects with Fabry Disease (MARVEL 2)

Protocol Numbers: FLT190-01 and FLT190-02

Regulatory Identifiers:
IND number: 024628
EudraCT number: 2018-002097-51 (FLT190-01), 2019-004645-32 (FLT190-02)
ClinicalTrials.gov number: NCT04040049 (FLT190-01), NCT04455230 (FLT190-02)

Development Phases: Phase 1/2 (FLT190-01) Long-term Follow-up (FLT190-02)	**Indication:** Fabry Disease

Coordinating Investigator: [redacted]

Trial Sites: Participants were treated at 2 sites (1 in Germany and 1 in the United Kingdom). A further 5 sites (1 each in Italy, Austria, United Kingdom, Denmark, and Germany) screened participants who were not subsequently treated.

Test Product, Dose, Mode of Administration, and Batch/Lot Numbers: FLT190, also known as AAV2/S3-FRE1-GLA-co, is a replication-incompetent single-stranded recombinant adeno-associated virus (AAV) vector composed of a single-stranded deoxyribonucleic acid genome packaged in an AAV-derived protein capsid. FLT190 was administered as a single intravenous (IV) infusion.

Two participants each received a single dose of FLT190 at 0.75×10^{12} vector genomes (vg)/kg and 1 participant received a single dose of FLT190 at 1.5×10^{12} vg/kg.

Batch/lot numbers of FLT190 were: 18C246-01FC and 141000021.

Trial Period:

FLT190-01 First participant (screened): 08 July 2019
FLT190-01 Last visit of last participant: 02 May 2023

FLT190-02 First participant (entered): 08 September 2020
FLT190-02 Last visit of last participant: 24 October 2023

Trial 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 alpha galactosidase A (α-Gal A). Despite the availability of enzyme replacement therapy (ERT) and pharmacological chaperone therapy (PCT), which can stabilize progression of Fabry disease, these therapies require constant infusions to maintain α-Gal A. Gene therapy with FLT190 was expected to deliver higher and consistent levels of α-Gal A, as seen in nonclinical studies. A single administration of FLT190 has the potential to improve the clearance of globotriaosylceramide (Gb3) from tissues and thereby significantly improve patient outcomes.

The first-in-human clinical trial (FLT190-01) aimed to investigate the safety of FLT190 and the potential to alter the disease phenotype through the endogenous production of α-Gal A following a single administration of FLT190. The long-term follow-up trial (FLT190-02) aimed to investigate the long-term safety and durability of α-Gal A expression in participants who had been previously treated with FLT190 in a clinical trial.

FLT190-01 Objectives and Endpoints:

Primary Objective: • To investigate the safety of systemic administration of FLT190 in adult males with Fabry disease.	**Primary Endpoint:** • Safety as assessed by the reporting of adverse events (AEs).
Secondary Objectives: • To investigate endogenous production of α-Gal A following systemic administration of FLT190 in adult males with Fabry disease. • To investigate the clearance of Gb3 and globotriaosylsphingosine (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 production of α-Gal A following systemic administration of FLT190.	**Secondary Endpoints:** Safety: • Safety as assessed by reporting of abnormal or change from Baseline findings from safety assessments including, laboratory assessments, vital signs, electrocardiogram (ECG), 24-hour Holter monitor, echocardiogram, cardiac magnetic resonance imaging (MRI), physical examination, and liver ultrasound. Efficacy: • Change from Baseline in Gb3 and 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 α-Gal A: • Immune response to the human α-Gal A transgene product was to be assessed by measurement of total and neutralizing antibodies up to Week 38 following systemic administration of FLT190. Shedding: • Clearance of vector genome in blood, urine, saliva, stool, and semen.

	Pharmacokinetic: • Change from Baseline in plasma α-Gal A activity and concentration at Week 12, 24, and 38. • Overall α-Gal A activity and concentration area under the curve (AUC) from Baseline to Week 38.
FLT190-02 Objectives and Endpoints:	
Primary Objective: • To assess the long-term safety of FLT190 in participants with Fabry disease.	**Primary Endpoints:** • The primary safety endpoint was to be assessed by the reporting of AEs. • Other safety endpoints were to be reviewed including: — Laboratory parameter abnormalities. — Vital signs, physical examination, liver ultrasound, and ECG abnormalities. — Cardiac MRI at 18 months post-FLT190 infusion.
Secondary Objectives: • To investigate the durability of endogenous production of α-Gal A enzyme.	**Secondary Endpoints:** • Change from Baseline in plasma α-Gal A expression over time (Baseline in this trial refers to the Baseline for the preceding FLT190 treatment trial). • Need for continuation or resumption of ERT or PCT after systemic administration of FLT190.
Trial Design: FLT190-01: FLT190-01 was a Phase 1/2, open-label, baseline-controlled, non-randomized, single-ascending dose trial of FLT190 in participants with Fabry disease. Participants underwent screening assessments for up to 18 weeks prior to treatment with FLT190. Treatment-eligible participants reported to the trial site on the day prior to receiving FLT190 for Baseline (Day -1) assessments. On Day 1, FLT190 was administered as a single dose, slow intravenous (IV) infusion into a peripheral vein, and the participant remained in the trial site for at least 8 hours following the end of the infusion, until the investigator deemed the participant as fit to be discharged. Participants were assessed at intervals over a 38-week post-treatment period. To monitor the shedding of vector genome sequences, participants were required to provide plasma, saliva, urine, stool, and semen samples. Contraception had to be used until three consecutive samples taken at separate visits (at least 1 week apart) were shown to be clear of vector genome sequences. This was a first-in-human trial and as such, an ascending-dose design was implemented to enable dose evaluation in a step-wise manner. The trial was planned to be conducted in 2 parts. In Part 1, up to 4 dose cohorts of vector were planned to be tested in a dose escalation scheme in previously treated patients (PTPs; defined as participants who were currently receiving either a licensed ERT or PCT and had been receiving the therapy at a stable dose for at least 12 months prior to FLT190 dosing). Eligible participants who were receiving ERT maintained their current treatment until the	

results from a minimum of 2 consecutive samples taken at least 4 weeks post-dosing showed α-Gal A expression higher than pre-dose trough α-Gal A expression, after which ERT could be withdrawn. Eligible participants who were receiving PCT had their treatment stopped prior to Baseline (Day -1) measurements. Based on the half-life of current approved PCT, a minimum 7-day washout period between final dosing of PCT and Day -1 was observed. Cohorts were planned to include between 2 and 3 PTPs each. For each participant, safety outcomes (as determined by AE reporting and laboratory tests) and α-Gal A expression were evaluated after dosing. Dependent on safety and α-Gal A expression, a decision was made on whether to recruit another participant into the cohort or to proceed to the next dose cohort. Dose escalation was overseen by an independent data monitoring committee (DMC).

An interim analysis was planned to be performed once dose escalation was complete in PTPs in Part 1 and all participants had at least 12 weeks of data following FLT190 administration. The intended aim of this interim analysis was to inform the recommended dose for Part 2 of the trial and for a future Phase 3 trial in PTPs.

In Part 2, a single cohort of 3 previously untreated patients (PUPs; defined as patients who had never previously received treatment with ERT or PCT [licensed or investigational]) were to be treated at the dose level selected from Part 1.

On completion of the trial, participants were invited to participate in the long-term, follow-up trial (FLT190-02) for a planned total of 5 years after dosing.

The trial was terminated early by the Sponsor due to discontinuation of the FLT190 program for commercial reasons after 3 participants had been treated in Part 1 of FLT190-01 (Part 2 did not take place). No interim analysis was performed.

<u>FLT190-02:</u>

FLT190-02 was a multicenter, long-term, follow-up trial of participants with Fabry disease who had undergone dosing with FLT190 in FLT190-01.

Participants were asked to provide consent for FLT190-02 prior to or upon completion of (or withdrawal from) FLT190-01. Consenting participants rolled over into FLT190-02 as soon as their participation in FLT190-01 had ended.

Data on safety and durability of α-Gal A expression were to be collected up to Month 60 (Year 5) post-treatment, with comparisons made to the participants' Baseline results prior to dosing with FLT190. All safety data (AEs and serious adverse events [SAEs]) were collected for the duration of the trial. Concomitant medication data were not collected, unless they were Fabry disease treatments (i.e., ERT or PCT) and/or immunosuppressive treatments for vector-associated immune AEs which were collected throughout the trial.

The trial was terminated early by the Sponsor due to discontinuation of the FLT190 program for commercial reasons.

This abbreviated Clinical Study Report presents the collated data from the two trials.

Number of Participants: Up to 12 PTPs were planned to be treated across up to 4 dose cohorts in Part 1 of FLT190-01. Three PUPs were planned to be treated in Part 2 at the dose selected in Part 1. At the end of FLT190-01, all participants who had been treated with FLT190 were invited to participate in the long-term follow-up trial (FLT190-02). There was no target sample size for FLT190-02; it was anticipated that fewer than 10 participants would be enrolled.

A total of 11 participants were screened for FLT190-01 and 3 participants were treated. All 3 treated participants entered FLT190-02. All screened participants from FLT190-01 and FLT190-02 were analyzed.

Participant Population: All participants were adult males with a previously confirmed diagnosis of classic Fabry disease with plasma α-Gal A activity less than 5% of normal.

Inclusion Criteria:

All participants must have fulfilled the following to be included in FLT190-01:

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 galactosidase A [GLA] mutation).
3. Plasma α-Gal A 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. eGFR ≥60 mL/min/1.73m^2 at Screening, per serum creatinine, using the Chronic Kidney Disease Epidemiology Collaboration equation.
6. <500 mg/g urine protein to creatinine ratio 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 ability 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, participants must have received either a licensed ERT or PCT for at least 12 months prior to FLT190 dosing, at a stable dose (participants receiving both ERT and PCT were ineligible). For inclusion in Part 2, participants must have never previously received treatment with either ERT or PCT (licensed or investigational).
11. Willingness to avoid strenuous exercise during the first 3 months after FLT190 dosing.

All participants must have fulfilled the following to be included in FLT190-02:

1. Participants who had previously received FLT190 (including those who may have required recommencement/initiation of ERT/PCT).
2. Participants able to give full informed consent and able to comply with all requirements of the trial including long-term follow-up for 60 months (5 years) post-treatment.

Exclusion Criteria:

Participants were not included in FLT190-01 if they met any of the following exclusion criteria:

1. Presence of GLA mutations leading to non-classical Fabry disease manifestation and any mutations that had not yet been classified.
   NOTE: Reference to the Fabry disease mutation database was used to determine classic and non-classical mutations (e.g., International Fabry Disease Genotype-Phenotype Database). If there was any uncertainty around the mutation classification, it was discussed with the 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. Participants with a history of chronic kidney disease, stages 3-5 (Kidney Disease: Improving Global Outcomes 2012 classification), documented in medical records for a minimum of 3 months.
5. Participants 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 alanine aminotransferase (ALT) or aspartate aminotransferase; or bilirubin >1.5 × upper limit of normal, during Screening.
8. Platelet count $<100 \times 10^9$/L.
9. Participants receiving warfarin or other anticoagulants interfering with the ability to perform renal or skin biopsies, or participants 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 or hepatitis B core antibody, or negative for hepatitis B surface antibody.
11. A positive serology test at Screening for hepatitis C antibody with a positive hepatitis C virus ribonucleic acid load assay, or were currently undergoing anti-viral therapy for hepatitis C.
12. A negative test at Screening for anti-varicella zoster virus immunoglobulin (Ig)G.
13. Either history of human immunodeficiency virus (HIV) infection, or a positive serology test at Screening for HIV.
14. A history of tuberculosis (TB), or a positive screening test for TB. The screening test may have been repeated once following an indeterminate result.
15. Participants who had received a live attenuated vaccination within 12 weeks prior to Screening or intended 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 [2nd or 3rd degree], supraventricular or ventricular arrhythmias).
19. Participants 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 or their excipients.

23. Participants who were assessed as having any contraindications to MRI. Including participants with ferromagnetic metallic implants, including pacing and defibrillator devices, nerve stimulators, and cochlear implants.
24. Participants who had a renal transplant.
25. Cytomegalovirus (CMV) IgG positive participants who were CMV polymerase chain reaction 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 have affected the participant's safety or could have interfered with the study assessments.
27. History of substance abuse including alcohol abuse or alcohol dependence.

(There were no exclusion criteria for FLT190-02.)

Statistical Methods: All planned analyses are described in the Statistical Analysis Plan (SAP), with the reduced list of outputs produced for this abbreviated Clinical Study Report following the early termination of the trial detailed in the SAP Addendum. All analyses were conducted using SAS® (SAS Institute Inc., Cary, NC, USA) statistical software version 9.4 or later.

All data collected from FLT190-01 and FLT190-02 are provided in listings, except data collected only for trial administrative purposes. Listings are presented by dose level and sorted by participant number and assessment date (and time), if applicable. Listings include data from both trials with the relevant trial number.

All endpoints were assessed using the Screened Set, which consisted of all participants who provided consent for FLT190-01 and FLT190-02.

All efficacy figures and listings considered all the data from both FLT190-01 and FLT190-02 for the Screened Set, unless specified otherwise. Gb3 and lysoGb3 in plasma and urine were listed, including change from Baseline.

AEs were coded using the Medical Dictionary for Regulatory Activities coding system (version 23.0) and listed by system organ class and preferred term. AEs were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 or assessed as mild, moderate, or severe. Treatment-emergent adverse events (TEAEs) were defined as any AEs that began or worsened (higher grading if events were CTCAE graded, or a more severe intensity) on or after the administration of FLT190. All AEs were included in a by-participant listing. Other safety data listed included, but were not limited to: laboratory evaluations, vital signs, 12-lead ECG, 24-hour Holter monitor, liver ultrasound, physical examination, and echocardiogram.

Summary of Results:

Demography and Baseline Characteristics

- All 3 treated participants were male, White, and Not Hispanic or Latino, with ages of 29, 45, and 46 years old at the time of screening in FLT190-01. The participants' heights were 174, 178, and 188 cm and weights were 55, 67.8, and 80.5 kg.

Disposition

- A total of 11 participants were screened for FLT190-01. Three participants were treated, which included: 2 participants treated with 0.75×10^{12} vg/kg FLT190 (Part 1, Cohort 1) and 1 participant treated with 1.5×10^{12} vg/kg FLT190 (Part 1, Cohort 2).

- Two of the treated participants completed FLT190-01 and the third treated participant was transferred early into FLT190-02 due to the Sponsor's early termination of FLT190-01. All 3 treated participants entered FLT190-02 and were subsequently discontinued due to the Sponsor's early termination of the trial.
- The duration of follow-up in the trial ranged from approximately 1 to 4 years.

Efficacy

- Participant 1, treated at 0.75×10^{12} vg/kg, had plasma α-Gal A activity levels plateau at around 0.7-1.0 nmol/h/mL and remain fairly stable at this level up to Month 42. Participant 2, treated with 0.75×10^{12} vg/kg, had plasma α-Gal A activity levels progressively rise to a peak at around Week 12, before stabilizing at around 3-4 nmol/h/mL up to Month 28. For Participant 3, treated with 1.5×10^{12} vg/kg, plasma α-Gal A activity levels reached a peak around Week 15 and subsequently progressively declined before seeming to stabilize by Week 34 at around 0.5 nmol/h/mL.
- Following FLT190 administration, Participant 1 experienced a rise from Baseline in plasma lyso-Gb3 after withdrawal of ERT, which was restarted at Week 6, and plasma lyso-Gb3 levels returned to Baseline levels by Week 24. A slow progressive increase in plasma lyso-Gb3 was seen in Participant 2 following ERT withdrawal. Participant 3, who remained on ERT throughout the trials, had post-dosing plasma lyso-Gb3 levels similar to Baseline until around Week 8, a brief increase to a peak at Week 16, followed by a return to a level similar to Baseline levels by around Week 22.
- No clinically significant eGFR, cardiac MRI, or brain MRI findings were reported.

Safety

- There were no deaths, dose limiting toxicities (DLTs) or AEs that led to early trial discontinuation.
- All 3 treated participants experienced at least 1 TEAE during FLT190-01, and 2 participants experienced at least 1 TEAE during FLT190-02.
- All 3 participants experienced TEAEs of myocarditis or TEAEs consistent with myocarditis (preferred terms: myocarditis, ECG ST segment elevation, troponin increased, and N-terminal prohormone brain natriuretic peptide [NT-pro BNP] increased) of mild to moderate severity. All TEAEs resolved without requiring specific treatment, and cardiac imaging and Holter ECG monitoring showed no evidence of new cardiac injury or arrhythmia, respectively.
- There were 13 FLT190-related TEAEs in 2 participants during FLT190-01; all were of mild to moderate severity. The most common FLT190-related TEAE was ALT increased.
- Two participants experienced 5 SAEs during FLT190-01 (myocarditis, transaminases increased, pyrexia, urinary tract infection, and ALT increased); 4 of 5 SAEs were considered FLT190-related.
- Two participants experienced 2 adverse events of special interest (AESIs) during FLT190-01 (transaminases increased and ALT increased); both were considered FLT190-related.
- No SAEs, AESIs, or FLT190-related TEAEs were reported during FLT190-02.
- All participants achieved vector clearance (at least three consecutive vector genome values of less than the lower limit of quantification) in saliva, semen, stool, plasma, and urine samples during FLT190-01.

Conclusions:

Efficacy Conclusions

- Administration of FLT190 led to an increase in plasma α-Gal A activity in all 3 participants. In 2 participants, levels remained above Baseline at the time of discontinuation of FLT190-02 (up to 4 years of follow-up), whilst in 1 participant, levels returned to near Baseline.
- The profile of α-Gal A expression was variable between participants, even those receiving the same weight-based dose.
- Expression of α-Gal A appeared durable to around 4 years after dosing.
- It is not possible to draw any conclusions about efficacy based on biomarkers (e.g., plasma lyso-Gb3) or clinical parameters due to the small sample size.

Safety Conclusions

- The safety profile of FLT190 was satisfactory, based on the data collected from 3 participants.
- There were 5 SAEs (4 of which were related to FLT190) reported during the trials.
- All 3 participants experienced TEAEs of myocarditis or TEAEs consistent with myocarditis (preferred terms: myocarditis, ECG ST segment elevation, troponin increased, and NT-pro BNP increased) of mild to moderate severity, which resolved with no evidence of persisting cardiac injury.
- There were no deaths, DLTs, or AEs leading to early discontinuation from the trials.

Publication References:

Not applicable.

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