FREELINE

ABBREVIATED CLINICAL STUDY REPORT SYNOPSIS

A Dose Confirmation Study of FLT180a (Adeno-associated Viral Vector Containing the Padua Variant of a Codon-optimized Human Factor IX Gene) in Adult Subjects with Hemophilia B

Protocol Number:	FLT180a-06
Investigational Medicinal Product:	FLT180a
Development Phase:	1/2
Trial Start Date:	First participant screened: 08 February 2022
Trial Completion Date:	Last visit of last participant: 11 June 2023
Sponsor:	Freeline Therapeutics Ltd Sycamore House, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2BP, UK
Sponsor Signatory/Sponsor's Responsible Medical Officer:	[redacted]
Coordinating Investigator:	[redacted] The Katharine Dormandy Haemophilia Centre, Royal Free Hospital, Pond Street, London, NW3 2QG, UK
Regulatory References:	EudraCT number: 2021-001079-18 ClinicalTrials.gov identification number: NCT05164471
Report Date:	23 April 2024 (Final v1.0)

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Name of Sponsor/Company: Freeline Therapeutics	**Individual Study Table Referring to Part of the Dossier** **Volume:** **Page:**	***(For National Authority Use Only)***
Name of Finished Product: FLT180a		
Name of Active Ingredient: FLT180a		

Protocol Title: A Dose Confirmation Study of FLT180a (Adeno-associated Viral Vector Containing the Padua Variant of a Codon-optimized Human Factor IX Gene) in Adult Subjects with Hemophilia B

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Development Phase: Phase 1/2 | **Indication:** Hemophilia B

Coordinating Investigator: [redacted] The Katharine Dormandy Haemophilia Centre, Royal Free Hospital, Pond Street, London, NW3 2QG, UK

Trial Sites: Six sites in the USA and UK.

Test Product, Dose, Mode of Administration, and Batch/Lot Numbers: FLT180a, also known as verbrinacogene setparvovec, is a recombinant adeno-associated virus (AAV) vector serotype S3, containing the Padua variant of a codon optimized human factor IX (FIX) gene.

FLT180a was administered as a single intravenous (IV) infusion.

The 3 participants in Cohort 1 received a single dose of FLT180a of 7.7×10^{11} vector genomes (vg)/kg with a dose cap at a participant weight of 90 kg (maximum dose = 69.3×10^{12} vg). The 3 participants in Cohort 2 received the same dose, per recommendations by the Data Monitoring Committee (DMC).

Batch/lot numbers of FLT180a were: 1855206 and 1908452.

Trial Period: First participant screened: 08 February 2022. Last visit of the last participant: 11 June 2023

Trial Rationale: Somatic gene therapy based on AAV vectors for the treatment of hemophilia B offers the potential for endogenous production of FIX after the transfer of a normal copy of the FIX gene; even small increases in FIX activity can have a large impact on symptom severity. The liver is a natural target for gene therapy of hemophilia B because FIX is normally produced in hepatocytes and recombinant AAV vectors with tropism for the liver have been developed.

Continuous synthesis of FIX by the host cells after a single administration of gene therapy offers a real opportunity to prevent bleeding episodes and eliminate the need for regular infusions. Data from the Phase 1/2 trial 15/0552 (NCT03369444; EudraCT 2017-000852-24) demonstrated a sustained FIX activity following a single infusion of FLT180a, suggesting the potential to eliminate the need for FIX replacement infusions. Nine out of 10 participants underwent phenotypic conversion from their

moderate to severe hemophilia B phenotype with the requirement for frequent infusions with recombinant FIX, to a milder or normal phenotype with stable levels of FIX in the upper mild hemophiliac range and above and without the requirement for administration of any FIX either prophylactically or for the treatment of bleeding events.

Objectives:

Primary Objective

- To assess the safety and tolerability of a single IV administration of FLT180a.

Secondary Objectives

- To describe the immune responses to the FIX transgene product and AAVS3 capsid protein following a single IV administration of FLT180a.
- To assess viral shedding in various body fluids following a single IV administration of FLT180a.
- To assess the long-term durability of response (FIX expression levels and protection from bleeding events) following a single IV administration of FLT180a.

Endpoints:

Safety Endpoints

- Treatment-emergent adverse events (AEs), AEs assessed by the investigator as related, serious adverse events (SAEs), SAEs assessed by the investigator as related, AEs leading to (early) trial discontinuation, deaths, and adverse events of special interest (AESIs).
- Abnormal or change from baseline findings for liver ultrasound or serum alpha-fetoprotein levels.
- FIX inhibitor levels.
- Clearance of vector genomes in plasma and semen.

Efficacy Endpoints

- FIX activity levels (central laboratory).
- Number of bleeding events over time up to Year 1.

Trial Design: This was a Phase 1/2, open-label, non-randomized, multicenter, dose confirmation, safety, and efficacy trial of FLT180a in adult male patients with severe (FIX activity <1%) or moderately severe (FIX activity 1% to 2% with severe bleeding phenotype) hemophilia B.

All participants came from the FLT-01 lead-in study (ECLIPSE), from which there was at least 6 months of data collected in a prospective manner detailing bleeding events and FIX consumption.

All eligible participants received a single dose of FLT180a. The dose was capped at a participant weight of 90 kg (maximum dose = 69.3×10^{12} vg). On Day 1, FLT180a was administered as a single-dose, slow IV infusion into a peripheral vein, and the participants were monitored closely.

The first 3 treated participants (Cohort 1) received a dose of 7.7×10^{11} vg/kg FLT180a. The DMC reviewed data from Cohort 1 after the 3 participants reached Day 21 to recommend whether to retain or modify the initial dose level for Cohort 2 according to prespecified criteria established via dose-response modelling. (A linear regression model of data from the prior Phase 1/2 trial [15/0552] demonstrated that Day 21 FIX activity data were predictive of Day 182 FIX activity by application of a scaling factor of 1.71 to Day 21 FIX values. Therefore, the Day 21 FIX activity data served as an early indicator of FIX activity on Day 182 [Week 26].) Following their review, the DMC recommended that 7.7×10^{11} vg/kg (the same dose as for Cohort 1) should be used to treat Cohort 2. Three participants were treated as part of Cohort 2.

All participants who received treatment were planned to be followed for safety and durability of response for 5 years following the administration of FLT180a; however, the trial terminated early with participants completing between 47 and 54 weeks of follow-up.

Number of Participants: Planned=up to 9; screened=7; treated=6; analyzed=6.

Participant Population: Adult males ≥18 and ≤65 years of age with hemophilia B with known severe or moderately severe FIX deficiency (≤2% of normal circulating FIX activity) for which continuous, stable, and adequate FIX prophylaxis was being taken.

Inclusion Criteria:

All participants must have fulfilled the following to be included in the trial:

1. Adult males ≥18 and ≤65 years of age.
2. Participants with hemophilia B with known severe or moderately severe FIX deficiency (≤2% of normal circulating FIX activity) for which the participant was on continuous stable, and adequate FIX prophylaxis.
3. Had acceptable laboratory values (per central laboratory).
    a. Hemoglobin ≥11g/dL
    b. Platelets ≥100,000 cells/µL
    c. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphate (ALP) ≤ upper limit of normal
    d. Serum albumin > lower limit of normal
    e. Total bilirubin ≤1.5 × upper limit of normal (except if caused by Gilbert's disease)
    f. Serum creatinine ≤2.0 mg/dL
4. Were able to give full informed consent and able to comply with all requirements of the trial including long-term follow-up.
5. Were willing to practice barrier contraception until at least three consecutive semen samples after vector administration were negative for vector sequences.
6. Had a level of neutralizing anti-AAVS3 antibodies below the limit of the pre-established clinical cut-off using an in vitro transduction inhibition assay within 4 weeks prior to FLT180a administration.
7. Had demonstrated ability to accurately, independently, and in a timely manner, enter bleed diary data during the lead-in study, as judged by the investigator.
8. At least 150 exposure days to FIX concentrates.
9. At least 6 months of satisfactory controlled prospective baseline data for bleeding events and FIX consumption from the FLT-01 lead-in study.

Exclusion Criteria:

Participants were not included in the trial if the participant had:

1. Demonstrated inability or unwillingness to comply with trial procedures or had a history of noncompliance.
2. Any history of alcohol or drug dependence.
3. Presence of neutralizing anti-human FIX antibodies (inhibitor; determined by the Nijmegen modified Bethesda inhibitor assay) at the time of enrolment or a previous history of FIX inhibitor.

4. Participants at high risk of thromboembolic events. This included:
   a. Participants with a history of arterial or venous thromboembolism (e.g., deep vein thrombosis, pulmonary embolism, non-hemorrhagic stroke, arterial embolus) and those with acquired or inherited thrombophilia, and/or
   b. Participants that had received anticoagulants including heparin during screening.
5. Evidence of advanced liver fibrosis (suggestive of or equivalent to METAVIR Stage 3), per one of the following diagnostic tests for liver fibrosis:
   a. FibroScan score ≥8.3kPa, or
   b. FibroTest/FibroSURE >0.48, or
   c. AST-Platelet Ratio Index >1
6. Used an investigational therapy within 60 days of enrolment.
7. Prior treatment with a gene transfer medicinal product.
8. Participants with active hepatitis B or C, and hepatitis B surface antigen, hepatitis B DNA or hepatitis C (HCV) RNA viral load positivity, or were currently on antiviral therapy for hepatitis B or C. Negative viral assays in two samples, collected at least 6 months apart, were required to be considered negative. Both participants who had cleared HCV spontaneously and those who had cleared HCV on antiviral therapy were eligible.
9. Serological evidence of human immunodeficiency virus-1, not controlled with anti-viral therapy and as evidenced by cluster of differentiation 4+ counts ≤200/μL.
10. Cytomegalovirus (CMV) IgG positive participants who were CMV polymerase chain reaction (PCR) positive at Screening.
11. A known coagulation disorder other than hemophilia B.
12. A history of uncontrolled cardiac failure, unstable angina, or myocardial infarction or other acute cardiac conditions requiring clinical management in the past 6 months.
13. A known or suspected intolerance, hypersensitivity, or contraindication to the investigational product excipients.
14. A known history of an allergic reaction or anaphylaxis to FIX products or known uncontrolled allergic conditions.
15. A known history of an allergy to corticosteroids or macrolides.
16. A known medical condition that would require chronic administration of corticosteroids (excluding topical formulations).
17. A planned surgical procedure within the next 12 months requiring prophylactic FIX treatment.
18. A known active severe infection (including documented coronavirus disease 2019 [COVID-19]) infection or any other significant concurrent, uncontrolled medical condition including, but not limited to, renal, hepatic, cardiovascular, hematological, immunological, gastrointestinal, endocrine, pulmonary, neurological, cerebral or psychiatric disease, malignancy or any other psychological disorder evaluated by the investigator to interfere with adherence to the protocol procedures or with tolerance to gene therapy.

Statistical Methods: All analyses are described in the Statistical Analysis Plan (SAP), with the reduced list of outputs to be produced for this abbreviated Clinical Study Report following the early termination of the trial detailed in the SAP Addendum.

Trial data were tabulated using data listings and summarized using descriptive statistics. FLT-01 lead-in data were included, as appropriate. Continuous variables were summarized using the number of observations, mean, standard deviation (SD), median, interquartile range (Q1, 25% quantile and Q3, 75% quantile), and range (minimum, maximum), and categorical variables were summarized using the number of observations and percentages, as appropriate.

All endpoints were assessed using the Full Analysis Set population, which consisted of all participants who entered the FLT-01 lead-in study and were subsequently dosed with FLT180a.

Efficacy data were listed but not tabulated. FIX activity levels were assessed using the one-stage activated partial thromboplastin time (aPTT) assay (at both central and local laboratories) and a chromogenic assay (at central laboratory only) and displayed graphically. The FIX response was derived for each participant based on the FIX activity level results from the central laboratory one-stage aPTT assay. Clinical responders were defined as participants with FIX activity level >40% and normalized responders were defined as participants achieving a FIX activity level between 50% to 147% at any post-baseline visit from Day 15 after dosing. Data for bleeding events and FIX concentrate consumption were collected from participant eDiaries and adjudication of bleeding events by investigators. Data for other analyses were listed and included immunogenicity assessments of FIX inhibitors and AAVS3 antibodies, and health resource utilization.

Adverse events were collected and coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 24.0. Tabulations included treatment-emergent adverse events (TEAEs), FLT180a-related AEs, SAEs, AESIs, and AEs leading to trial discontinuation. TEAEs were defined as AEs that commenced on or after the first dose of FLT180a or that increased in severity if present prior to first dose. Other safety data were listed but not tabulated, including but not limited to: laboratory data, electrocardiogram (ECG), vital signs, physical examinations, liver ultrasound, viral shedding, corticosteroids, immunosuppressants, and tacrolimus levels, and modified absolute risk score (ARS).

Summary of Results:

This trial was terminated early by the Sponsor due to commercial reasons.

Demography and Baseline Characteristics

- All 6 participants in the trial were male (100.0%), predominantly White (66.7%) and Not Hispanic or Latino (83.3%), with a mean (SD) age of 29.0 (11.66) years and a mean (SD) body weight of 81.40 (9.519) kg.

Disposition

- Of the 6 participants, 3 participants were treated in Cohort 1 and 3 participants were treated in Cohort 2. All participants received 7.7×10^{11} vg/kg FLT180a. All participants were discontinued due to Sponsor termination of the trial and completed between 47 and 54 weeks of follow-up.

Efficacy

- All participants achieved a clinical response (FIX level of >40%) within 1 to 5 weeks following administration of FLT180a and maintained their status as a clinical responder for 7 to 49 weeks.
- Investigator adjudication of bleeding events reported 17 true bleeding events in 3 participants, with 1 participant experiencing 12 minor spontaneous true bleeds and three moderate spontaneous true bleeds.

Safety

- During the trial, there were no deaths, SAEs, or AEs that led to trial discontinuation.
- All participants had at least one TEAE and FLT180a-related TEAE. The most commonly reported TEAEs were alanine aminotransferase increased (83.3%), and COVID-19, arthralgia, and headache (50.0% each). The most commonly reported FLT180a-related TEAE was alanine aminotransferase increased (83.3%).
- AESIs were reported in 5 participants and included alanine aminotransferase increased in 5 (83.3%) participants and factor IX deficiency in 2 (33.3%) participants.
- There were no SAEs associated with laboratory abnormalities or laboratory abnormalities graded as severe in the trial. There were no participants with abnormal vital signs during the trial.

- Clearance of vector genomes from semen was reached by all participants during the trial. Viral shedding in plasma was less than the lower limit of quantification or less than the limit of detection in 5 of 6 participants at trial termination while 1 participant achieved clearance at Week 26.

Conclusions:

Efficacy Conclusions

- All participants achieved a clinical response to FLT180a treatment, which was maintained for varying durations, and with the majority of participants experiencing ≤1 bleed during the trial.

Safety Conclusions

- FLT180a was well tolerated. There were no deaths, SAEs, or events leading to trial discontinuation.

Publication References: None

Report Version & Date: Final v1.0 23 April 2024