2 SYNOPSIS

NAME OF COMPANY: University College London	**INDIVIDUAL STUDY SYNOPSIS**	
NAME OF FINISHED PRODUCT: FLT180a	**INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER**	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF ACTIVE INGREDIENT(S): FLT180a (AAV2/S3-HLP2-Ti-FIXco1)	**Volume:** **Page:**	
Title of Study: A Phase I/II, Open-label, Multicentre, Ascending Single Dose, Safety Study of a Novel Adeno--associated Viral Vector (FLT180a) in Patients With Haemophilia B.		
Study Sites: Nine clinical sites in the United Kingdom and Ireland screened patients for the study; 6 sites enrolled patients into the study.		
Studied Period: Date of First Observation: 19 December 2017 Date of Last Observation: 11 August 2020	Phase of Development: I/II	

Objectives:

Primary Objectives

The primary safety objective was to assess the safety of systemic administration of FLT180a in adults with haemophilia B (HB) in up to 3 different dose cohorts.

The primary efficacy objective was to assess factor IX (FIX) levels after systemic administration of FLT180a, at the terminal dose level.

Secondary Objectives

- To investigate the endogenous production of FIX after systemic administration of FLT180a at up to 3 different dose cohorts.
- To investigate the effectiveness of a single administration of FLT180a on annualised bleeding rate (ABR) and exogenous FIX consumption.
- To assess the immune response to the FIX transgene product after systemic administration of FLT180a.
- To assess viral shedding in various body fluids after systemic administration of FLT180a.

Exploratory Objectives

- To assess the immune response to the adeno-associated virus S3 (AAV-S3) capsid proteins after systemic administration of FLT180a.
- To investigate the impact of endogenous production of FIX on functional status and disability, quality of life (QoL), physical activity, haemophilia health status, joint health, and health resource utilisation in HB.

Methodology:

This was a Phase I/II, open-label, multicentre, ascending single-dose, safety study of FLT180a in patients with severe (FIX activity <1%) or moderately severe (FIX activity 1% to 2% with severe bleeding phenotype) HB. Up to 24 patients were planned to be enrolled; however, the study was terminated early (on 20 October 2020) after 10 patients had been enrolled because of changes to the clinical development plan and recruitment difficulties due to the COVID-19 pandemic.

Patients who provided consent to participate and had historical data on bleeding and FIX consumption documented from the previous 3 years' medical notes were screened for eligibility in this study. During the screening period, patients completed a diary to prospectively record ongoing bleeding events and FIX consumption. Patients were monitored through a comprehensive schedule of safety assessments at outpatient visits for 26 weeks.

On completion of the study, patients are to be followed for 15 years under a separate long-term follow-up protocol.

Patients who provided consent to participate in this study underwent screening assessments up to 52 weeks before study Day 0 (FLT180a infusion). Due to the risk of bleeding in this patient population, a washout from the patient's FIX concentrate regimen was not mandated. The investigator was to demonstrate, from the patient's medical records, a documented FIX activity level of <1% for severe patients or <2% for moderately severe patients. If (at the investigator's discretion) a FIX concentrate washout was undertaken during the screening period, a minimum of 5 days' washout was required.

Treatment-eligible patients reported to the study site on the day before receiving the gene therapy infusion (Day -1). On Day 0, FLT180a was administered as a single dose, slow intravenous (IV) infusion into a peripheral vein. The patient remained in the study centre for ≥12 hours and until the investigator deemed the patient fit to be discharged. The first 2 patients treated at each dose level remained at the study centre for 24 hours after infusion before discharge.

Patients who were on prophylactic therapy with FIX concentrates remained on their usual dosing schedule and were closely monitored for FIX activity levels after screening and administration of FLT180a. If FIX activity levels ≥3% were reached, then prophylaxis was held pending a repeat analysis within a period of 72 hours. If the FIX activity levels were ≥3% atthat time, then prophylaxis was stopped with continued/regular assessment of FIX activity levels and occurrence of spontaneous bleeding.

Patients were required to undergo study evaluations at intervals over the 26-week, post-treatment period. These evaluations took place either at the study infusion site or at their normal haemophilia treatment centre. To monitor for shedding of vector genome (vg) sequences, patients were required to provide plasma, saliva, urine, stool, and semen samples until the results of 3 successive samples were clear.

This was a first-in-human study; therefore, an ascending-dose design was implemented to enable dose evaluation in a step-wise manner. Three dose cohorts of vector (low, intermediate, and high) were tested in the dose escalation. Two patients were tested at each dose level with an additional patient added in the event of a dose limiting toxicity (DLT) (2 + 1 design). Dose escalation occurred provided there was no more than 1 DLT at any dose cohort and if the resulting FIX activity failed to reach the target level. A reduction of the dose level within a cohort occurred if the FIX activity exceeded defined levels to reduce the risk of exceeding the normal physiological range. A dose reduction occurred when the 2 + 1 design was applied at that new dose level within the cohort. At the discretion of the Sponsor after advice from the trial management group (TMG) and independent data monitoring committee (DMC), additional patients were to be added to any cohort to ensure adequate characterisation of either safety or the FIX response before dose escalation/reductions. The Sponsor, TMG, and DMC planned to select the terminal dose level based on the patient FIX activity levels with the aim of ensuring most patients reached a FIX activity level within normal limits and in the absence of DLTs; the terminal dose level was planned to be expanded to 14 patients, but never reached. This design minimised the number of patients who would need to be dosed at suboptimal levels while allowing evaluation of safety with the option to expand a group on observation of DLTs. An extended 6-week interval was observed between the first and second patient on study to monitor for any unanticipated, delayed adverse events (AEs). Subsequently, when dose escalation was ongoing, the study mandated a minimum 4-week interval between patients during which time efficacy and safety was reviewed before a decision to dose the next patient.

The main risk in this study was a dose-dependent, asymptomatic increase in the serum alanine aminotransferase (ALT) level associated with a decline in FIX levels, suggesting a loss of transduced hepatocytes. In this study, all patients were given a take-home pack of immunosuppressants (prednisolone only) to be taken under the direction of the investigator, which allowed rapid intervention if transaminase elevations were observed. In addition, during the anticipated critical time period all patients were to receive a course of immunosuppressants (prednisolone, methylprednisolone, and tacrolimus) beginning at the Week 3 visit or Week 4 visit in line with the relevant protocol version active at the time.

The main efficacy endpoint was based on an analysis of the proportion of patients achieving a clinical or normalised FIX response at 26 weeks. A clinical FIX response was defined as achieving a FIX activity of 5% to 150% of normal. Five percent had been selected as the threshold for a clinical FIX response because using gene therapy in patients with HB to increase FIX activity from <1% to 5% had previously been shown to lead to a highly clinically significant improvement in ABRs and exogenous factor consumption. A normalised FIX response was defined as achieving a FIX activity level in the normal range (50% to 150%). The normal range had been selected as the threshold level for a normalised FIX response because reaching this level was expected to modify the patient phenotype from severe at study start to normal at which point patients would not be expected to experience spontaneous bleeds.

The choice of a 26-week endpoint was based on previous experience with AAV gene therapy for HB in which patients achieved steady-state FIX levels by 16 weeks after gene therapy. Based on this, it was anticipated that the patients' FIX activity would reach a stable level by 26 weeks, thus this was an appropriate point at which to measure activity.

Number of Patients: A total of 17 patients were screened, of whom 10 met eligibility criteria and were enrolled into the study. Two patients were enrolled at each of the 6×10^{11} vg/kg, 2×10^{12} vg/kg, and 1×10^{12} vg/kg dose levels and 4 patients at the 1.3×10^{12} vg/kg dose level.

All 10 patients who received FLT180a completed the study and consented to participate in the 15-year long-term follow-up study.

Diagnosis and Criteria for Inclusion:

Inclusion Criteria:

To participate in the study, patients must have met the following criteria:

1. Adult males, ≥18 years of age.
2. Confirmed diagnosis of HB defined as one of the following:
    a. Documented severe FIX deficiency with plasma FIX activity of <1% of normal, or
    b. Moderately severe FIX deficiency with plasma FIX activity level between ≥1% and ≤2% and a severe bleeding phenotype defined by one of the following:
        i. On prophylaxis for a history of bleeding, or
        ii. On-demand therapy with a history of 4 or more bleeding episodes/year on average over the past 3 years, or
        iii. Evidence of chronic haemophilic arthropathy (pain, joint destruction, and loss of range of motion).
3. Provided full informed consent and complied with all requirements of the study including 15-year, long-term follow-up.
4. Was willing to practice barrier contraception until at least 3 consecutive semen samples after vector administration were negative for vector sequences.
5. Had a lack of neutralising anti-AAV-S3 antibodies using an in vivo transduction inhibition assay within 4 weeks of vector administration.
6. Had at least 150 exposure days to FIX concentrates.

Exclusion Criteria:

To participate in the study, patients must not have met any of the following criteria:

1. Presence of neutralising antihuman FIX antibodies (inhibitor, determined by the Bethesda inhibitor assay) at the time of enrolment or a previous history of FIX inhibitor.
2. Patients at high risk of thromboembolic events (high-risk patients included those with a history of arterial or venous thromboembolism [eg, deep vein thrombosis, pulmonary embolism, nonhaemorrhagic stroke, arterial embolus] and those with acquired thrombophilia including conditions such as atrial fibrillation).
3. Use of investigational therapy for haemophilia within 30 days before enrolment.
4. Patients with active hepatitis B virus (HBV) or hepatitis C virus (HCV), and hepatitis B surface antigen or HCV RNA viral load positivity, respectively, or currently on antiviral therapy for HBV or HCV. Negative viral assays in 2 samples, collected at least 6 months apart, were required to be considered negative. Both natural clearers and those who have cleared HCV on antiviral therapy were eligible.
5. Serological evidence of HIV-1.
6. Evidence of liver dysfunction (persistently elevated ALT, aspartate aminotransferase, bilirubin >1.5×upper limit of normal).
7. Platelet count $<50\times10^{9}$/L.
8. Uncontrolled glaucoma, diabetes mellitus, or hypertension.
9. Malignancy requiring treatment.
10. Patients with uncontrolled cardiac failure, unstable angina or myocardial infarction in the past 6 months.

11. Poor performance status (World Health Organization score >1).
12. Previous treatment with any gene transfer medicinal product.
13. Known or suspected intolerance, hypersensitivity, or contraindication to the investigational product and noninvestigational medicinal products or their excipients.
14. Planned major elective surgery before the end of study (EOS).
15. Current or relevant history of a physical or psychiatric illness or any medical condition that in the opinion of the investigator could affect the patient's safety or interfere with the study assessments.
16. Cytomegalovirus (CMV) immunoglobulin G positive patients who are CMV polymerase chain reaction (PCR) positive at screening.

Test Product, Dose, Mode of Administration, and Batch Number(s):

FLT180a was provided as a concentrate for solution for infusion in a 5-mL (of FLT180a) vial.

FLT180a was given as a single-dose, slow IV infusion. The planned dose escalation scheme was as follows:

- Cohort 1 (low dose): 6×10^{11} vg/kg
- Cohort 2 (intermediate dose): 2×10^{12} vg/kg
- Cohort 3 (high dose): 4×10^{12} vg/kg

The dose level within a cohort was to be reduced based on observed FIX activity levels:

- Cohort 2 (intermediate dose): 2×10^{12} vg/kg could have been reduced to:
  - 1.5×10^{12} vg/kg
  - 1.3×10^{12} vg/kg
  - 1×10^{12} vg/kg
  - 8×10^{11} vg/kg
- Cohort 3 (high dose): 4×10^{12} vg/kg could have been reduced to:
  - 3×10^{12} vg/kg

During the dose escalation phase of the study, a review of FIX data at a dose of 1.3×10^{12} vg/kg was suggestive of the impact of body weight on expression levels.

The FIX activity levels at 1×10^{12} vg/kg dose had stabilised around the bottom end of the normal range at steady state (approximately 50%), and with the 2×10^{12} vg/kg dose peak FIX activity levels have exceeded the normal range (>150%). As a result, at the meetings, the TMG and DMC requested the addition of intermediary doses to further characterise the dose response and allow patients to achieve the target range for FIX activity levels as specified in the protocol. The protocol was amended to include 2 additional doses in the medium dose cohort at 1.3×10^{12} vg/kg and 1.5×10^{12} vg/kg. Given the high FIX expression levels observed in 1 patient, the TMG agreed that reducing the dose to 1.3×10^{12} would allow safety and efficacy to be assessed cautiously going forward, which was ratified by the DMC. The actual 4 dose levels of FLT180a studied were:

- 6×10^{11} vg/kg
- 2×10^{12} vg/kg
- 1×10^{12} vg/kg and
- 1.3×10^{12} vg/kg

Investigational Medicinal Product Batch Number: 17-089/IM011

Duration of Study Participation:

Maximum duration of patient involvement in the study:

- Planned duration of screening period: Up to 52 weeks
- Planned duration of follow-up period after infusion: 26 weeks
- Planned duration of long-term follow-up: 15 years (under a separate extension protocol)

Criteria for Evaluation:

Efficacy: Baseline FIX activity was established for all patients. A trough level of FIX activity was established during the screening period. Samples at the various time-points were analysed at a central laboratory (efficacy) and local laboratory (efficacy and safety) as indicated. Local blood samples were taken Day -1, Day 0 (pre-dose), Day +2, Day +4, 3 times per week between Week 1 and Week 5, 2 times per week between Week 6 and Week 25 and once in Week 26/EOS. Blood samples for assessment of FIX antigen were also drawn in accordance with the schedule of assessments. Bleeding episodes were entered into the patient's diary and included: number, location, aetiology (eg, spontaneous, traumatic), and total units of FIX concentrate required to resolve the bleed. For each dose of FIX concentrate administered information regarding the concentrate, dose given in total international unit, the reason for administration (prevention, bleeding episode) and frequency of administration were recorded in the patient diary.

Safety: The following sites were examined: head, neck, ears, nose, throat, eyes, chest, lungs, heart, abdomen, skin, and lymph nodes. The following systems were assessed: musculoskeletal and neurological. The patient's height (screening only) and weight were also measured. Waist circumference (cm), hip circumference (cm), neck circumference (cm), and bioimpedance were measured at screening and Day -1 only. Laboratory tests were performed at a central/local laboratory to assess safety: haematology, chemistry, coagulation screen, liver function tests, and FIX activity level. Blood samples for assessment of FIX neutralising antibody development (inhibitor), AAV-S3 antibodies, for reactivation of hepatitis, tacrolimus levels, and CMV testing were also drawn in accordance with the schedule of assessments. Measurements of vital signs (blood pressure, pulse, temperature, and respiratory rate), liver ultrasound, and 12-lead electrocardiogram (ECG) were conducted in accordance with the schedule of assessments.

Patients were questioned in a general way at each study visit to establish whether AEs had occurred since the previous visit. Additionally, the investigator evaluated other collected data (eg, patient diaries, questionnaires, clinical evaluations) to ascertain whether an AE had occurred. AEs were collected from the time initial informed consent was signed until Week 26.

Other Variables: Plasma, saliva, urine, stool, and semen samples for PCR of vg were taken in accordance with the schedule of assessments for infusion week. These collections occurred until there were 3 consecutive negative samples.

Health economic assessments, QoL, disability, physical activity, haemophilia health status, and health resource utilisation were also assessed.

Exploratory: Joint health/function, mononuclear cells (immune studies), mononuclear cells (research), research plasma, FIX activity research plasma, immune response research plasma were assessed.

Study Endpoints:

Primary Endpoints

Safety

Safety was assessed by reporting AEs according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Efficacy

- The proportion of patients achieving clinical FIX response at Week 26, at the terminal dose level. A clinical FIX response was defined as achieving a FIX activity of 5% to 150%.
- The proportion of patients also achieving normalised FIX response at Week 26, at the terminal dose level. A normalised FIX response was defined as achieving FIX activity in the normal range (50% to 150%).

Secondary Endpoints

Safety

Safety was assessed by reporting abnormal or change from baseline findings from routine safety assessments including laboratory assessments, vital signs, ECG, physical exam, and liver ultrasound.

Endogenous FIX Production

- The proportion of patients achieving FIX activity at or above 5%, 15%, 30%, 40%, 50%, and 70% but no more than 150% of normal, at each scheduled visit.

- The proportion of patients achieving FIX activity at or above 5%, 15%, 30%, 40%, 50%, 70%, and 150% of normal, at each scheduled visit. Absolute change from baseline in FIX activity.

Haemostatic Effectiveness

- Change from baseline in ABR.
- Change from baseline in FIX Concentrate Consumption.

To ensure enough time has elapsed for the patient to have endogenous FIX activity to protect the patient from spontaneous bleeding episodes, the calculation period for haemostatic effectiveness was from Day 15 inclusive.

Immune Response

- Immune response to the FIX transgene product (ie, development of inhibitors) was assessed by measurement of the level of inhibitors.

Shedding

- Clearance of vector genomes in plasma, saliva, urine, stool, and semen.

Exploratory Endpoints

Haemostatic Effectiveness

- Exploration of the correlation between FIX levels and bleeding events over time.

Immune Response

- Immune response to the AAV-S3 capsid was assessed by measurement of the S3 neutralising antibody titre.
- T-cell responses to AAV-S3 capsid in peripheral blood mononuclear cells.

Disability Status

- Change from baseline in World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0) score.

Physical Activity

- Change from baseline in Haemophilia Activities List (HAL) 2005.

Health-related Quality of Life

- Change from baseline in the EuroQoL 5-dimension 5-level (EQ-5D-5L) and the Haemophilia Quality of Life (Haem-A-QoL) score.

Haemophilia Health Status

- Change from baseline in the Patient Reported Outcomes, Burdens and Experiences (PROBE) score.

Assessment of Joint Health/Function

- Change from baseline in the Haemophilia Joint Health Score (HJHS).

Health Resource Utilisation

- Number of haemophilia-related medical appointments and medical activities.
- Number of visits at site.
- Number of emergency room visits.
- Number of hospitalisations related to haemophilia.
- Length of hospital stay.
- Number of days lost from education or work by patients and caregivers due to bleeding episodes.
- Number of physiotherapy sessions, specialist consultations and appointments with professional caregivers.

Statistical Methods:

The statistical analysis plan (SAP) described the general guidelines for analysis as well as the following items:

- SAS version 9.4 was used.
- Disposition was presented for all patients in the Screened Set by dose level and overall.
- Demographics, baseline characteristics, and safety endpoint summaries were presented for all patients in the full analysis set, by dose level and overall.

- Efficacy summaries were presented for all patients in the Full Analysis Set (FAS), by dose level and overall.
- The total number of patients in the dose level was used as the denominator for percentage calculations, unless stated otherwise in the table shell.
- Continuous variables were summarised using the number of observations (n), mean, SD, median, minimum, and maximum. Categorical variables were summarised using n, frequency, and percentages of patients.
- Data from all patients, including screen failures, were entered into the database and included in the disposition listings. Screen failure data were included at the end of the appropriate listings and labelled 'Screen Failures'.
- All safety data were entered into the patient listings.
- In general, the listings were presented by dose level and sorted by patient number and assessment date (and time) if applicable.
- Multiple assessments at a given time point (planned, repeat) were not included in summary tables unless specified otherwise but were included in the listings. If there were multiple results at a given visit, eg, a repeated lab test within a visit, the earliest value was used.
- In general, unscheduled visit data were listed but not included in the summary tables by time point. However unscheduled visit data were used for the last observation carried forward (LOCF) methodology for the primary efficacy analysis. Also, for shift tables, unscheduled data were included to identify the worst-case post FLT180a dose.

Key definitions, visit windows, and the methods used for missing data and subgroup analysis are described in the SAP.

SUMMARY OF RESULTS

Efficacy Results:

- The study was terminated early, and the study objectives could not be met.
- Although only 2 of the 4 patients at the 1.3×10^{12} vg/kg dose level formally met the pre-specified definition of a clinical FIX response (FIX level at 6 months 5% to 150%). The other 2 patients exhibited FIX levels >150%. Within this dosing group, treatment with a single dose of FLT180a thus led to FIX activity in the normal range in 2 patients and slightly above the normal range in the other 2 patients.

	1.3×10^{12} vg/kg (N=4)
Week 26/EOS	
Number of Patients Assessed	4
Clinical Responders, n (%) (95% CI)	2 (50.0) (6.8, 93.2)
Normalised Responders, n (%) (95% CI)	2 (50.0) (6.8, 93.2)

- Four of the 10 patients achieved FIX activity within the normal range (≥50 to ≤150%), 7 patients formally met the pre-specified definition of a clinical response (≥5 to ≤150%) and 9 achieved FIX activity ≥40%.
- FLT180a treatment was effective in reducing ABR. Postdose (Day 15 to Week 26), the ABR decreased to a mean (SD) of 1.09 (SD: 1.84). FLT180a treatment also led to dramatic reduction in FIX concentrate consumption. At baseline, the patients had an annualised total units of FIX concentrate consumption of 226026.32 IU per year. No patient required any FIX concentrate during the post dose period.
- There was no evidence of FIX inhibitor. The FIX inhibitor titre results were not measured accurately in 9 patients because their basal FIX activity was >20%.
- Clearance of vector genomes was studied in various body fluids. Vector genomes were generally below the quantifiable limit from all matrices studied within the first month post dose. The fastest clearance was reported in saliva (median time 1.71 weeks) followed by in urine (2.07 weeks), semen (2.07 weeks), plasma (3.14 weeks), and stool (3.78 weeks).
- Overall, a single administration of FLT180a showed favourable results of haemostatic effectiveness and improving disability status, physical activity, joint/health function, health resource utilisation, and health-related QoL.

Safety Results:

- Although the study was terminated early and the study objectives could not be met, the safety data demonstrate that treatment with FLT180a was safe and well tolerated during the study.
- The most commonly reported serious treatment-emergent AEs (TEAEs) during the study were ALT increased, and transaminases increased as is typical in this therapeutic area.
- All TEAEs reported during the study were Grade 1 to Grade 3 in severity and no patient died due to TEAE. The most frequently reported TEAEs were ALT increased, diarrhoea, fatigue, headache, arthralgia, muscle spasm, dyspepsia, and tremor.

Conclusions:

- The study objectives could not be met because the study was terminated early.
- Overall, this study demonstrated that treatment with FLT180a was safe and well tolerated during the study period.
- By the end of the study period, each of 4 patients who received 1.3×10^{12} vg/kg dose achieved a FIX activity within the normal range or slightly higher.
- FLT180a treatment led to marked improvement in endogenous FIX production leading to significant reduction in ABR and dramatic reduction in exogenous factor consumption.
- Complete clearance of FLT180a vector from all studied matrices (eg, plasma, urine, stool, saliva, semen) occurred quite rapidly, within the first month post-dosing. FLT180a vector cleared predominantly through saliva.
- A single administration of FLT180a showed favourable results in terms of haemostatic effectiveness and improving disability status, physical activity, joint/health function, health resource utilisation, and overall health-related QoL in adult patients with HB.
- The UCL 15/0552 FIX-GT Trial was terminated prematurely on the 20 October 2020. The decision was made in the context of the COVID-19 pandemic and the challenges it had posed to gene therapy clinical trials, as well as information from the regulators on the requirement to submit data for the marketing authorisation application, from patients dosed with FLT180a product manufactured under the long-term commercial process. The clinical development plan for FLT180a was adjusted as a result, with early termination of this Ph1/2 and further trials with FLT180a, generated under the long-term commercial process. The planned enrolment (n=24) was not achieved, rendering the resulting dataset (n=10) less complete than envisioned for this first-in-human phase 1/2 trial.

Date of Report: 10 Sep 2021