PRS record reference: **NCT03369444**

Trial Title**: A Phase I/II, Open label, Multicentre, Ascending Single Dose, Safety Study of a Novel Adenoassociated Viral Vector (FLT180a) in Patients With Haemophilia B**

This document contains the following study related documents:
15/0552 - Protocol Version 8.1, 21 Apr 2020 (final)

Full title of trial:	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
Short title:	A Factor IX Gene Therapy Study (FIX-GT)
Version and date of protocol:	Final Version 8.1 21 Apr 2020
Previous Versions:	Version 1.0 12 Apr 2017 Version 2.0 26 Sep 2017 Version 3.0 14 May 2018 Version 3.1 22 May 2018 Version 4.0 21 Nov 2018 Version 5.0 12 Mar 2019 Version 6.0 30 Apr 2019 Version 7.0 19 Jul 2019 Version 7.1 07 Aug 2019 Version 8.0 29 Feb 2020
Sponsor:	University College London (UCL)
Sponsor protocol number:	15/0552
Funder:	Freeline Therapeutics Ltd.
Funder Reference:	FLT180a-01
EudraCT no:	2017-000852-24
IND #:	018311
Active IMP	FLT180a
Phase of Trial:	Phase I/II
Site(s):	International multicentre study

Chief Investigator:
Pratima Chowdary
The Katharine Dormandy Haemophilia and Thrombosis Centre
Royal Free Hampstead NHS Trust
Royal Free Hospital, Pond Street
Hampstead, London NW3 2QG

Sponsor Representative:
Joint Research Office
UCL
Gower Street
London WC1E 6BT

Signatures

Investigator's Acknowledgement

I have read the protocol for 15/0552.

Title: A Phase I/II, Open label, Multicentre, Ascending Single Dose, Safety Study of a Novel Adeno-associated Viral Vector (FLT180a) in Patients with Severe Haemophilia B.

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

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 authorisation from the sponsor. It is, however, permissible to provide the information contained herein to a patient in order 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, 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

Address: _____

Chief Investigator:

Dr Pratima Chowdary [redacted]

Sponsor Representative:

[redacted]

1 Emergency Contact Information

In the event of a serious adverse event (SAE), the investigator must complete the Clinical Trial SAE Form and ensure its transmission within 24 hours to the contract research organisation (CRO) pharmacovigilance department. Applicable fax numbers and e-mail addresses can be found on the SAE form (sent under separate cover).

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

[redacted]

Or if unavailable, contact the central out-of-hours group at [redacted]

Contents

2 List of Abbreviations

AAV	Adeno-associated virus
AE	Adverse Event
AR	Adverse Reaction
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATIMP	Advance Therapy Investigational Medicinal Product
CBC	Clinical Biotechnology Centre
CMV	Cytomegalovirus
CRO	Contract Research Organisation
CRP	C-reactive protein
DLT	Dose-limiting toxicity
DMC	Data monitoring committee
EC	Ethics Committee
eCRF	Electronic case report form
ECG	Electrocardiogram
EOS	End of Study
EU	European Union
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transpeptidase
GMP	Good Manufacturing Practice
GTMP	Gene Therapy Medicinal Product
FIX	Factor IX
HAL	Haemophilia Activities List
HB	Haemophilia B
HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus

hFIX	Human Factor IX
HIV	Human immunodeficiency virus
HJHS	Haemophilia Joint Health Score
[redacted]	[redacted]
ICF	Informed consent form
ICH	International Conference on Harmonisation
IMP	Investigational medicinal product
IRB	Institutional Review Board
IU	International unit
IV	Intravenous(ly)
LFT(s)	Liver function test(s)
MRI	Magnetic Resonance Imaging
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIMP	Non-investigational medicinal product
PCR	Polymerase chain reaction
PI	Principal Investigator
PROBE	Patient Reported Outcomes, Burdens and Experiences
QoL	Quality of life
QP	Qualified person (for release of trial drug)
rcAAV	Replication competent adeno-associated virus
SAE	Serious adverse event
SAR	Serious adverse reaction
SAP	Statistical Analysis Plan
SUSAR	Suspected unexpected serious adverse reaction
TAT	Thrombin-antithrombin complex
TEAE	Treatment Emergent Adverse Event
TMG	Trial Management Group
TSC	Trial Steering Committee
UCL	University College London
US	United States

vg	Vector genomes
WFH	World Federation of Hemophilia
WHODAS	World Health Organisation Disability Assessment Schedule

3 Trial Personnel

Chief Investigator	Pratima Chowdary [redacted]
Sponsor's representative UCL Joint Research Office	[redacted]
CRO Project Manager Syneos Health, LLC	[redacted]

4 Summary

Protocol number: 15/0552	**Drug**: FLT180a (AAV2/S3-[redacted]-Ti-FIXco1)

Title of the 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

Number of patients:

Up to 24 patients may be enrolled into the study; the actual number of patients will depend on the criteria for dose escalation.

14 patients will be enrolled at the terminal dose level.

Investigator(s): This is a multicentre study.

Site(s) and region(s):

The study will be conducted at sites across Europe, Africa and North America.

Clinical phase: I/II

Objectives:

Primary:

Safety

To assess the safety of systemic administration of FLT180a in adults with haemophilia B (HB) at up to 3 different dose cohorts.

Efficacy

To assess Factor IX (FIX) levels following systemic administration of FLT180a, at the terminal dose level.

Secondary:

- To investigate the endogenous production of FIX following 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 and exogenous FIX consumption.
- To assess the immune response to the FIX transgene product following systemic administration of FLT180a.
- To assess viral shedding in various body fluids after systemic administration of FLT180a.

Exploratory:

- To assess the immune response to the adeno-associated virus (AAV)-S3 capsid proteins following systemic administration of FLT180a.

Following a single administration of FLT180a:

- To investigate the impact of endogenous production of FIX on functional status and disability in HB.
- To investigate the impact of endogenous production of FIX on quality of life (QoL) in HB.
- To investigate the impact of endogenous production of FIX on physical activity in HB.
- To investigate the impact of endogenous production of FIX on haemophilia health status in HB.
- To investigate the impact of endogenous production of FIX on joint health in HB.
- To investigate the impact of endogenous production of FIX on health resource utilisation in HB.

Rationale:

Haemophilia B is an X-linked recessive bleeding disorder that affects approximately 1 in 30,000 males. The disorder results from a defect in the *f9* gene and is characterised by frequent spontaneous bleeding into joints and soft tissues which, without adequate treatment, causes a chronic debilitating arthropathy.

Current treatment for HB in the Western World involves intravenous (IV) infusions of plasma-derived or recombinant factor concentrates at the time of a bleed (on-demand therapy) or as regular prophylactic infusion of factor concentrate. On-demand treatment is effective at arresting haemorrhage but cannot prevent chronic damage that ensues after a bleed. In severely affected patients, prophylactic therapy to prevent bleeding episodes can greatly reduce such bleeding when plasma FIX levels are maintained at or above only 1% of normal.[1] The relatively short half-life of standard FIX necessitates frequent IV administration of factor concentrates (2-3 times a week) for prophylaxis against haemorrhage, which is invasive, inconvenient, and

[1] Ljung RC. Prophylactic infusion regimens in the management of hemophilia. Thromb.Haemost. 1999; 82:525-530.

problematic for children. Even with extended half-life (EHL) factor concentrates frequent infusions are required for the life time of the patient.

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. In addition, current experience suggests that the level of efficacy seen with gene therapy is likely to be superior to that seen with prophylactic treatment and, when considering the most recent data, may even offer the prospect of a functional cure with a lack of haemorrhage even in response to trauma or surgery.

This study aims to characterise the safety of a new gene therapy (FLT180a) and investigate its potential to alter the disease phenotype from severe to mild/normal through the endogenous production of FIX following a single administration of vector.

Investigational product, dose, and mode of administration:

FLT180a will be given as a single dose, slow IV infusion.

The dose escalation scheme proposed:

- Cohort 1 (low dose): 6 x 10^{11} vector genomes (vg)/kilogram (kg) (of body weight)
- Cohort 2 (intermediate dose): 2 x 10^{12} vg/kg
- Cohort 3 (high dose): 4 x 10^{12} vg/kg

Based on the observed FIX response, the dose level within a cohort may be reduced to avoid exposing patients to levels that exceed the normal physiological range, see Protocol Section 12.3.1.

Methodology:

This is 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 1-2% with severe bleeding phenotype) HB. Patients who provide consent to participate in this study will be screened for eligibility and will have historical data on bleeding and FIX consumption documented from the previous 3 years' medical notes. During the screening period patients will complete a diary to prospectively record on-going bleeding events and FIX consumption.

On the day prior to infusion (Day -1), the patient will attend the investigational site and final eligibility assessments will be conducted. On Day 0, FLT180a will be administered as a single dose, slow IV infusion into a peripheral vein, and the patient will be monitored closely. Subject to satisfactory results from safety evaluations, the patient will be discharged from the investigational site but will continue to be monitored through a comprehensive battery of safety assessments at outpatient visits for a period of 26 weeks, following which the patient will enter a period of long-term follow-up conducted under a separate extension protocol.

Up to 3 dose cohorts of vector (low, intermediate, and high) will be tested in the dose escalation. Two patients will be tested at each dose cohort with an additional patient added in the event of a dose-limiting toxicity (DLT) (2 + 1 design). Safety outcomes (as determined by adverse event [AE] reporting and laboratory tests) and results of FIX activity will be overseen by a trial management group and a data monitoring committee consisting of independent medical experts. Dose escalation may occur provided there is no more than 1 DLT at any dose cohort and if the resulting FIX activity fails to reach the target level. The goal for FIX response is to maximise the number of patients who achieve a FIX activity in the range 70-150% whilst minimising the risk of overshooting the normal physiological range. In order to reduce the risk of overshooting the normal physiological range, dose reduction within a dose cohort may occur if FIX activity in the initial patients in that dose cohort exceeds levels predefined in the protocol. Where a dose reduction occurs the 2+1 design will apply at that new dose level within the cohort. Dose escalation/reductions will be overseen by the trial management group and data monitoring committee. Additional patients may be added to a dose cohort to ensure adequate characterisation of any DLT, safety issues not meeting DLT criteria, or the FIX response at the request of the trial management group and data monitoring committee and at the discretion of the Sponsor.

All patients will be provided with a take-home pack of immunosuppressants which will only be used at the direction of the investigator and liver function tests will be monitored intensively post infusion. From the week 3 visit, all patients will take a course of immunosuppressants. At all other times, if patients develop liver inflammation, they will be instructed by the investigator to initiate treatment with a short course of immunosuppressants.

The Sponsor, trial management group and data monitoring committee will select the terminal dose level based on patient FIX activity levels with the aim of ensuring the majority of patients will reach a FIX activity within normal limits in the absence of dose-limiting AEs. This terminal dose level will be expanded to 14 patients.

On completion of the study, patients will be followed for 15 years under a separate extension protocol.

Inclusion and exclusion criteria:

Inclusion criteria:

1. Adults 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. Able to give full informed consent and able to comply with all requirements of the trial including 15-year long-term follow-up;
4. Willing to practice barrier contraception until at least three consecutive semen samples after vector administration are negative for vector sequences;
5. Lack of neutralising anti-AAV-S3 antibodies using an *in vivo* transduction inhibition assay within 4 weeks of vector administration;
6. At least 150 exposure days to FIX concentrates.

Exclusion criteria:

1. Presence of neutralising anti-human 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 would include those with a history of arterial or venous thromboembolism (e.g. deep vein thrombosis, pulmonary embolism, non-haemorrhagic stroke, arterial embolus) and those with acquired thrombophilia including conditions such as atrial fibrilation);
3. Use of investigational therapy for haemophilia within 30 days before enrolment;
4. Patients with active hepatitis B or C, and HBsAg or HCV RNA viral load positivity, respectively, or currently on antiviral therapy for hepatitis B or C. Negative viral assays in 2 samples, collected at least 6 months apart, will be required to be considered negative. Both natural clearers and those who have cleared HCV on antiviral therapy are eligible.;
5. Serological evidence of HIV-1;
6. Evidence of liver dysfunction (persistently elevated alanine aminotransaminase, aspartate aminotransferase, bilirubin >1.5 x upper limit of normal);
7. Platelet count <50 x 10^9/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. Prior treatment with any gene transfer medicinal product;
13. Known or suspected intolerance, hypersensitivity or contraindication to the investigational product and non-investigational medicinal products or their excipients;
14. Planned major elective surgery prior to the end of trial.
15. Current or relavant history of a physical or psychiatric illness or any medical condition that in the opinion of the investigator could affect the patients safety or interfere with the study assessments.
16. CMV IgG postive patients who are CMV PCR positive at screening.

Maximum duration of patient involvement in the study:

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

Endpoints and statistical analysis:

Endpoints:

Primary endpoints

Safety

Safety as assessed by the reporting of AEs according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

Efficacy

The following primary endpoints will be analysed:

- The proportion of patients achieving clinical FIX response at Week 26, at the terminal dose level. A clinical FIX response is defined as achieving 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 is defined as achieving FIX activity of in the normal range (50-150%).[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, 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 annualised bleeding rate.
- Change from baseline in FIX concentrate consumption.

In order 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 will be from day 15 inclusive.

Immune response

Immune response to the FIX transgene product (i.e., development of inhibitors) will be assessed by measurement of the level of inhibitors.

Shedding

Clearance of vector genomes in plasma, urine, saliva, 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 will be 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 score.

Physical activity

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

Health-related quality of life

Change from baseline in the EQ-5D-5L and the Haem-A-QoL score.

Haemophilia health status

Change from baseline in the 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 emergency room visits.

Number of hospitalisations related to haemophilia.

Length of hospital stay.

[2] https://www.wfh.org/en/page.aspx?pid=643

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 analysis:

Statistical analysis plan

All study data will be listed and all relevant data will be tabulated and summarised by dose cohort and overall. A Statistical Analysis Plan (SAP) will be written and finalised prior to database lock. This plan will give a detailed description of all summaries and analyses that will be presented.

Demographic and baseline characteristics

Continuous variables will be summarised using number of observations, mean and standard deviation, median, and minimum and maximum values. Categorical values will be summarised using number of observations and percentages. Medical history will be summarised using number of observations and percentages of patients reporting each category.

Primary endpoint

Safety

Adverse events will be coded using CTCAE version 5.0. Frequency of treatment-emergent AEs (TEAEs) will be calculated for each body system and preferred term, and by dose cohort, for number of events and number of patients reporting the event. The severity of the TEAEs and the relationship to study medication will be summarised for each body system and preferred term by dose level.

Efficacy

The FIX response will be derived for each patient based on the FIX activity level measured at the central laboratory. Clinical FIX response is defined as achieving FIX activity of 5-150% and normalised FIX response is defined as achieving FIX activity in the normal range (50-150%).

The two primary endpoints will be analysed on the full analysis set. The proportion of patients achieving clinical FIX response at Week 26, will be summarised for the patients who received the terminal dose level. For the primary analysis, last-observation-carried-forward (LOCF) methodology will be used to impute any missing FIX value at Week 26, by the last non-missing FIX value. For the primary analysis, FIX response will be assessed whether or not the patient is receiving or has been receiving immunosuppressants due to transaminitis outside the period of prophylactic immunosuppressant treatment. The proportion of patients also achieving normalisation will be summarised in a similar manner.

Secondary endpoints

Safety

Descriptive statistics (number of observations, mean, standard deviation, minimum, median and maximum values) will be calculated for clinical laboratory tests at applicable visits. Changes from baseline will also be presented. For laboratory tests, abnormal values will be flagged in the data listings. In particular, patients achieving FIX activity above 150% of normal will be summarised by dose and overall.

Vital signs (systolic and diastolic blood pressure, temperature, and pulse) and physical examination will be summarised by dose cohort using appropriate descriptive statistics. Continuous variables will be summarised using number of observations, mean, standard deviation, minimum, median, and maximum values. Categorical values will be summarised using number of observations and percentages.

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 will be summarised by dose and overall.
- The proportion of patients achieving FIX activity at or above 5%, 15%, 30%, 40%, 50%, 70% and 150% of normal, at each scheduled visit will be summarised by dose and overall.
- Change from baseline in FIX activity as a percentage of normal values will be summarised in tabular and graphical format for each patient, by dose and overall.

Haemostatic effectiveness

Annualised bleeding rate

The number of breakthrough bleeding episodes (spontaneous and traumatic) following FLT180a infusion will be annualised and compared with the patient's own baseline bleeding history (the annualised mean of 3 years of historical bleeding records, and where possible, prospective data collected during the screening period will be used).

Factor IX concentrate consumption

The dose (international unit [IU]/kg) of factor concentrate used overall and by type of bleeding episode (i.e., spontaneous or traumatic) and location of bleeding episode (i.e., joint, soft tissue, or muscle) will be summarised with descriptive statistics. The total units of annualised factor consumption will be calculated and compared with

the patient's own baseline factor concentrate history (the annualised mean of 3 years' historical factor concentrate consumption records, and where possible, prospective data collected during the screening period will be used).

In order 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 will be from day 15 inclusive to the date of completion of the last diary. More details will be provided in the SAP.

Immune response

FIX

Immune response to the FIX transgene product (i.e., development of inhibitors) will be assessed by measurement of the level of inhibitors.

Descriptive statistics (number of observations, mean, standard deviation, minimum, median and maximum values) will be calculated for immune response laboratory tests at applicable visits.

Shedding

Clearance of vector genomes in plasma, saliva, urine, stool, and semen will be summarised. The time to unquantifiable result by body fluid will be summarized and listed.

Table 1: Schedule of Assessments

Procedure	Week	Day					Week*									
	-52 to -1[A]	-1[B]	0	+1	+2	+4	1	2	3	4	5	6	7	8	9	10
Visit Window							+ / - 1 day									
Informed Consent	X	For Days -1 to +4 please see Table 2: Detailed Schedule of Assessments for Infusion Week														
Demographics and Medical History	X															
Bleeding History (incl. Target Joint Assessment)	X															
Prior and Concomitant Medications	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
Vital Signs[D]	X						X	X	X	X	X	X	X	X	X	X
12-lead ECG	X							X		X				X		
Adverse Event Assessment	X						X	X	X	X	X	X	X	X	X	X
Joint Evaluation (HJHS)	X															
Liver Ultrasound[E]	X															
AAV Antibody Screen[F]	X[G]															
FIX Antigen	X															
FIX Genotype[H]	X															
HBV, HCV, HIV CMV Screen[I]	X															
Baseline FIX Activity[J]	X															
FIX Activity Trough	X															
Diary Completion for Bleeding Events and FIX Consumption	→															→
QoL (EQ-5D-5L and Haem-A-QoL), Disability (WHODAS 2.0), Physical Activity (HAL 2005), Haemophilia Health Status (PROBE) and Health Resource Utilisation[K]	X															
Liver Function Test (Local)[L, M]							X[N]	X[N]	X[N]	X[N]	X[N]	X[O]	X[O]	X[O]	X[O]	X[O]
FIX Activity Level (Local)[M]							X[N]	X[N]	X[N]	X[N]	X[N]	X[O]	X[O]	X[O]	X[O]	X[O]
Haematology, Chemistry incl. CRP, Coagulation Screen (Local)[P]							X	X	X	X	X	X	X	X	X	X
Haematology, Chemistry incl. CRP, Coagulation Screen (Central)	X						X	X	X	X	X	X	X	X	X	X
Liver Function Test (Central)	X						X	X	X	X	X	X	X	X	X	X
FIX Activity Level (Central)[F]							X	X	X	X	X	X	X	X	X	X
FIX Inhibitor Level	X						X	X	X			X			X	
AAV-S3 Antibody Titre[F]							X	X				X				
Mononuclear Cells (Elispot)							X	X	X	X	X	X	X	X	X	X

Procedure	**Week**	**Day**					**Week***									
	-52 to -1[A]	**-1[B]**	**0**	**+1**	**+2**	**+4**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Visit Window							**+ / - 1 day**									
Mononuclear Cells (Research) - Optional		For Days -1 to +4 please see Table 2: Detailed Schedule of Assessments for Infusion Week														
Research Plasma Samples - Optional							X	X	X	X	X	X	X	X	X	X
FIX Activity Research Plasma Samples							X			X						
Immune Response Research Plasma Samples							X[Q]	X[Q]	X[Q]	X[Q]	X[Q]	X[Q]	X[Q]	X[Q]	X[Q]	X[Q]
Prophylactic Immunosuppressants[R]									– –	– – –	– – –	– – –	– – –	– – –	– – –	–▶
Test for Reactivation of Hepatitis[S]										X		X		X		X
Test for CMV[T]									X	X	X	X	X	X	X	X
Test for Tacrolimus Level[U]									X	X	X	X	X	X	X	X
PCR of Vector Genomes in Plasma, Saliva, Urine, Stool and Semen							X[V]	X[V]	X[V]	X[V]	X[V]	X[V]	X[V]	X[V]	X[V]	X[V]

Procedure	**Week***															
	11	**12**	**13[W]**	**14**	**15[W]**	**16**	**17[W]**	**18[W]**	**19[W]**	**20**	**21[W]**	**22[W]**	**23[W]**	**24[W]**	**25[W]**	**26/EOS[X]**
Visit Window	**+ / - 1 day**		**+ / - 3 days**													
Informed Consent																
Demographics and Medical History																
Bleeding History (incl. Target Joint Assessment)																
Prior and Concomitant Medications	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
Vital Signs[D]	X	X		X		X				X						X
12-lead ECG																X
Adverse Event Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Joint Evaluation (HJHS)																X
Liver Ultrasound[E]																X
AAV Antibody Screen[F]																
FIX Antigen		X														X
FIX Genotype[H]																
HBV, HCV, HIV CMV Screen[I]																
Baseline FIX Activity[J]																
FIX Activity Trough																
Diary Completion for Bleeding Events and FIX Consumption	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
QoL (EQ-5D-5L and Haem-A-QoL), Disability (WHODAS 2.0), Physical Activity (HAL 2005), Haemophilia Health Status (PROBE) and Health Resource Utilisation[K]																X
Liver Function Test (Local)[L, M]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X
FIX Activity Level (Local)[M]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X[O]	X
Haematology, Chemistry incl. CRP, Coagulation Screen (Local)[P]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Haematology, Chemistry incl. CRP, Coagulation Screen (Central)	X	X		X		X				X						X
Liver Function Test (Central)	X	X		X		X				X						X
FIX Activity Level (Central)[F]	X	X		X		X				X						X
FIX Inhibitor Level		X				X				X						X
AAV-S3 Antibody Titre[F]		X														X
Mononuclear Cells (Elispot)	X	X		X		X				X						X

Procedure	Week*															
	11	12	13[W]	14	15[W]	16	17[W]	18[W]	19[W]	20	21[W]	22[W]	23[W]	24[W]	25[W]	26/EOS[X]
Visit Window	+ / - 1 day		+ / - 3 days													
Mononuclear Cells (Research) - Optional																X
Research Plasma Samples - Optional	X	X		X		X				X						X
FIX Activity Research Plasma Samples																X
Immune Response Research Plasma Samples	X[Q]	X[Q]		X[Q]		X[Q]				X[Q]						X
Prophylactic Immunosuppressants[R]	----	----	----	----	----	----	----	----	----	---►						
Test for Reactivation of Hepatitis[S]		X		X		X		X		X						
Test for CMV[T]	X	X	X	X	X	X	X	X	X	X						
Test for Tacrolimus Level[U]	X	X	X	X	X	X	X	X	X	X						
PCR of Vector Genomes in Plasma, Saliva, Urine, Stool and Semen	X[V]	X[V]		X[V]		X[V]				X[V]						X[V]

Abbreviations: AAV = adeno-associated virus; CMV = cytomegalovirus; CRP = C-reactive protein; ECG = electrocardiogram; EOS = end of study; FIX = Factor IX; HAL = haemophilia activity list; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; HJHS = Haemophilia Joint Health Score; PCR = polymerase chain reaction; QoL = quality of life; WHODAS = World Health Organisation Disability Assessment Schedule.

* Week 1 visit is Day +7, Week 2 visit is Day +14, Week 3 visit is Day +21, Week 4 visit is Day +28 etc.

[A] The screening period can be up to 52 weeks to allow for controlled prospective collection of bleeding event and FIX consumption data via the patient diary. A patient can move forward to Day -1 assessments and dosing as dosing slots are available. There is no minimum time frame for the data collection. ALL screening assessments should be completed in a timely manner (~2 weeks) following consent to confirm patient eligibility. The patient should be contacted ~every 4 weeks through the screening period to check on diary completion and collect details on any AEs. A repeat AAV Antibody screen is mandated within 4 weeks of dosing. If the screening period for a patient is longer than 16 weeks then a repeat of the central laboratory screening bloods (FIX Antigen/ HBV, HCV, HIV, CMV Screen/ Haematology, Chemistry incl. CRP, Coagulation Screen/ Liver Function Test/ FIX Activity Level/ FIX Inhibitor Level) will be required within 4 weeks of dosing and the patient reported outcomes should also be repeated at Day -1.

[B] The Day -1 assessments can be conducted as early as Day -3 for logistical reasons, if required.

[C] Height (screening only) and weight (screening) will also be measured. Waist circumference (cm), hip circumference (cm), neck circumference (cm) and bioimpedance will be measured at screening.

[D] Vital signs include pulse, blood pressure.

[E] Dependent on results of liver ultrasound further investigations including fibroscan/MRI/elastography may be carried out.

[F] Two aliquots will be taken at each timepoint.

[G] A negative assay (transduction inhibition assay) outcome must be documented within 4 weeks of dosing. Repeat testing may be indicated due to the long screening window.

[H] Blood sample to be taken and sent to the central laboratory for testing if not already documented in the medical records.

[I] HCV antibody testing followed by HCV RNA load. HCV RNA viral load only indicated for patients with history of HCV and positive HCV antibody test. CMV IgG testing followed by CMV PCR. CMV PCR only indicated for a positive CMV IgG.

[J] Established following 5 days washout or from documented medical records.
[K] For Health Resource Utilisation at Screening a 6-month history will be collected and at EOS all Health Resource Utilisation since Screening (or Day -1) will be collected.
[L] Albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase, aspartate aminotransferase.
[M] Frequency to be increased in response to an upward trend in LFTs and in line with guidance in section 9.4.
[N] Three times per week. The tests should be as evenly spaced through the week as possible, for example: Monday, Wednesday and Friday. 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).
[O] Twice weekly. The tests should be as evenly spaced through the week as possible, for example: Monday and Thursday or Tuesday and Friday. On days when other clinic assessments are not required these samples may be taken at study site or at an alternative location (e.g. patient's home).
[P] Complete blood count with differentials and platelets. Chemistry incl. CRP to include sodium, potassium, phosphate, blood urea nitrogen or urea, serum creatinine (and estimated GFR), CRP. Coagulation screen to include prothrombin time and activated partial thromboplastin time.
[Q] To be drawn only during the course of investigation or treatment of breakthrough transaminitis.
[R] A weight based immunosuppressant regimen will be initiated at the Week 3 visit. For details see section 9.4.
[S] Local testing. Only required for patients with history of Hepatitis B or C, commencing at Week 4 and continuing every 2 weeks until prophylactic immunosuppressant regimen completed. Tests should be undertaken to coincide with use of immunosuppression 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).
[T] Local testing. For patients positive for CMV (IgG) at screening weekly CMV PCR testing for the duration of the immunosuppressant regimen. Tests should be undertaken to coincide with use of immunosuppression 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 alternative location (e.g. patient's home). Management guidelines in the case of CMV reactivation can be found in Appendix 2.
[U] Local testing. Tacrolimus levels should be tested at each blood draw until levels are in the therapeutic 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 3.
[V] Three times within 7-10 days following vector infusion then weekly until 3 consecutive samples are negative.
[W] Blood draws may be taken either at study site or at alternative location (e.g. patient's home). The intention should be for at least one of the two blood draws per week to be conducted at study site.
[X] In the event of patient discontinuation / withdrawal every effort should be made to complete week 26/end of study procedures.

Table 2: Detailed Schedule of Assessments for Infusion Week

Procedure	Day -1[a]	Day 0 (Infusion Day)																	Day		
		Pre-dose[b]	Timepoint (minutes) during Infusion					Timepoint (hours) from End of Infusion													
			0	+15	+30	+45	+60	+1	+2	+3	+4	+5	+6	+8	+10	+12	+16	+20	+1	+2	+4
Window	**- 2 days**			**+/- 5 mins**				**+/- 10 mins**						**+/- 15 mins**							**+/- 1 day**
Informed Consent[c]	X																				
Physical Examination[d]	X																	X	X	X
Vital Signs[e]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X[f]	X[f]	X	X	X
12-lead ECG	X																	X		
QoL (EQ-5D-5L and Haem-A-QoL), Disability (WHODAS 2.0), Physical Activity (HAL 2005), Haemophilia Health Status (PROBE) and Health Resource Utilisation[g]	X[h]																			
FLT180a Administration			X	----------			→														
Diary Completion for Bleeding Events and FIX Consumption	→																				→
Prior and Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Procedure	Day -1[a]	Day 0 (Infusion Day)																	Day		
		Pre-dose[b]	Timepoint (minutes) during Infusion					Timepoint (hours) from End of Infusion													
			0	+15	+30	+45	+60	+1	+2	+3	+4	+5	+6	+8	+10	+12	+16	+20	+1	+2	+4
Window	**- 2 days**			**+/- 5 mins**				**+/- 10 mins**						**+/- 15 mins**							**+/- 1 day**
Local Lab Tests																					
Haematology, Chemistry incl. CRP, Coagulation Screen[i]	X	X												X					X	X	X
Liver Function Test[j]	X	X												X					X	X	X
FIX Activity Level	X	X																		X	X
Central Lab Tests																					
Haematology, Chemistry incl. CRP, Coagulation Screen	X																			X	X
Liver Function Test	X																				
FIX Activity Level[k]	X	X																			
AAV Antibody Screen[k]	X																				
Mononuclear Cells (Elispot)	X																				
Mononuclear Cells (Research) - Optional	X																				
Research Plasma Sample - Optional	X																				
Immune Response Research Plasma Sample	X																				
PCR of Vector Genomes in Plasma, Saliva,	X																			X[l]	X[l]

Procedure	Day -1[a]	Day 0 (Infusion Day)																Day		
		Pre-dose[b]	Timepoint (minutes) during Infusion					Timepoint (hours) from End of Infusion												
			0	+15	+30	+45	+60	+1	+2	+3	+4	+5	+6	+8	+10	+12	+16	+20	+1	+2	+4
Window	**- 2 days**			**+/- 5 mins**				**+/- 10 mins**						**+/- 15 mins**							**+/- 1 day**
Urine, Stool and Semen																					

Abbreviations: AAV = adeno-associated virus; CRP = C-reactive protein; ECG = electrocardiogram; PCR = polymerase chain reaction.

[a] The Day -1 assessments can be conducted as early as Day -3 for logistical reasons, if required.
[b] Approximately 1 hour before infusion.
[c] The 'pre-infusion' dosing stage informed consent may occur prior to day -1.
[d] Weight (Day -1) will also be measured. Waist circumference (cm), hip circumference (cm), neck circumference (cm) and bioimpedance will be measured at Day -1.
[e] Vital signs from day -1 to day +4 include pulse, blood pressure, respiration rate and temperature.
[f] 16 and 20 hour timepoints are only required for the first two patients in each cohort.
[g] For Health Resource Utilisation at Day -1 all Health Resource Utilisation since Screening will be collected.
[h] Only to be completed if patients had been in the screening period for more than 16 weeks.
[i] Complete blood count with differentials and platelets. Chemistry incl. CRP to include sodium, potassium, phosphate, blood urea nitrogen or urea, serum creatinine (and estimated GFR), CRP. Coagulation screen to include prothrombin time, activated partial thromboplastin time.
[j] Albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase, aspartate aminotransferase.
[k] Two aliquots will be taken at each timepoint.
[l] Three times within 7-10 days following vector infusion then weekly until 3 consecutive samples are negative.

5 Introduction

5.1 Background

Haemophilia B (HB) is an X-linked recessive bleeding disorder that affects approximately 1 in 30,000 males. This disorder results from a defect in the Factor IX (*f9*) gene, which encodes a serine protease, critical for appropriate fibrin clot formation. Approximately 50% of individuals with HB have a severe disorder characterised by functional FIX levels of less than 1% of normal (<1 U/dL = 50 ng/mL). An additional “moderately severe” patient group also exists that is characterised by FIX levels between 1-2% whilst retaining the severe bleeding phenotype. Clinically the disease is characterised by frequent spontaneous bleeding into joints and soft tissues which, without adequate treatment, causes a chronic debilitating arthropathy. Spontaneous bleeding into closed spaces such as the brain occurs in a significant proportion of patients with catastrophic manifestations including death. Current treatment for HB in the Western World may be either on demand treatment or prophylactic treatment. On-demand treatment involves intravenous (IV) infusions of plasma-derived or recombinant factor concentrates at the time of a bleed. This is highly effective at arresting haemorrhage but cannot totally abrogate chronic damage that ensues after a bleed. In severely affected patients, bleeding episodes can be dramatically reduced with prophylactic treatment when plasma FIX levels are maintained at or above only 1% of normal by regular administration of FIX protein concentrates.[1] The relatively short half-life of standard FIX concentrates requires infusions to be frequent at 2-3 times a week. Even extended half-life (EHL) FIX concentrates require regular infusions. The need for regular infusions is invasive, inconvenient, and highly problematic for children. Central lines are often required in children which may cause significant morbidity due to catheter-related complications.

The cost of prophylactic treatment for an adult is in excess of $150,000 per year, a significant cost burden for all healthcare systems even within the developed world. In the context of haemophilia worldwide, the cheaper plasma-derived FIX concentrates, if used for treatment alone rather than prophylaxis, still remain unaffordable by the majority of people worldwide who have haemophilia B.[2] Formation of neutralising antibodies (inhibitors) which occur in 3% of HB patients resulting in poor response to factor concentrates, often with fatal outcomes, is a rare but significant complication of protein replacement therapy. Additionally, the psychological burden associated with the constant but unpredictable risk of bleeding in this chronic disorder is, perhaps, not fully appreciated. Therefore, replacement therapy with FIX products continues to be less than ideal both in the context of the developed as well as the developing world.

These unmet needs have fuelled interest in gene therapy of HB which has several fundamental advantages over other single-gene disorders. The clinical manifestations represent a simple cause and effect relationship which is attributable to the lack of a single gene product that circulates in minute amounts in the plasma. In addition, tightly regulated control of gene expression is not essential since a wide range of FIX is expected to be beneficial and nontoxic. The availability of animal models including FIX-knockout mice[3,4,5] and HB dogs[6,7] has facilitated extensive preclinical evaluation of gene therapy strategies. Perhaps the most important single

aspect of HB that makes it such an excellent target for gene therapy is the fact that even a small rise (1% of physiological levels) in circulating clotting factor would have a significant beneficial therapeutic effect which could transform the lives of patients with severe haemophilia. Small changes in FIX can be easily assessed using both clinical and laboratory evaluation. Continuous synthesis of FIX by the host cells after gene therapy provides a real opportunity to prevent bleeding episodes rather than simply treating the bleeds after they have occurred. Additionally, the risks and the inconvenience of regular infusion of protein concentrates would be avoided following gene therapy. Importantly endogenous synthesis of FIX within hepatocytes after liver-targeted delivery of vector may also lead to immunological tolerance to the transgene product thereby potentially reducing the risk of neutralising FIX antibody formation.[8,9,10] Finally, and crucially, endogenously synthesised FIX is maintained at a constant level after gene transfer, thus avoiding the “saw tooth” pattern produced by intermittent infusion with its inherent troughs, where break through bleeding is most likely to occur.

The results from gene therapy studies conducted to date offers the prospect of sustained FIX expression in excess of levels that can be achieved with exogenous prophylactic treatment. The achievement of superior efficacy suggests there is potential for a functional cure with a lack of haemorrhage even in response to trauma or surgery.

5.2 Nonclinical Data

The overall nonclinical development programme consists of *in vitro* and *in vivo* pharmacodynamic studies and single-dose Good Laboratory Practice-compliant toxicology/biodistribution studies in Rhesus Macaques.

In the text of this document, reference is made to FLT180 and FLT180a. FLT180a is the proposed investigational medicinal product and is an evolution of FLT180 which was the construct on which initial pre-clinical testing was carried out. The only difference between FLT180 and FLT180a is that FLT180a incorporates a change to the codon optimisation that was used in FLT180. The codon optimisation is in exons 2 to 8 of the *f9* gene and represents a change of <20% of the overall sequence. The capsid, promotor, intron, exons 1, poly A tail and the amino acid sequence of the factor IX produced are all unchanged and are identical to FLT180. Because the change does not affect the transgene product and only affects a small part of the overall vector sequence, the change is not expected to affect product quality attributes other than protein expression levels. On this basis, non-clinical work carried out on FLT180 is considered to be highly supportive of FLT180a.

Please refer to the FLT180a Investigator Brochure for full details of the non-clinical data including pharmacodynamics, toxicology and biodistribution.

5.3 Clinical Data

Currently a number of gene therapy medicinal products for HB are in development. These products are building on the work conducted by Nathwani et al, where 10 patients were treated with a first-generation AAV gene therapy.[11] In this study, a single IV infusion of AAV2/8-LP1-hFIXco resulted in an increase in plasma FIX activity from baseline values that ranged from

less than 1% of the normal value to steady-state levels of 1 to 6% of the normal value in all 10 patients. Patients lacked antibodies to the capsid that was used in the study and response was linked to the dose patients received. Among patients receiving a low (2 x 10^{11} vg/kg) or intermediate (6 x 10^{11} vg/kg) dose of vector, the increase in FIX activity was modest (1 to 3% of the normal value). In contrast, remarkably consistent FIX expression (mean, 5.1 ±1.7% of the normal value) was observed in the 6 patients receiving the high dose of vector. Following treatment AAV8-mediated FIX expression remained stable over a period of >6 years, in keeping with the stability of transgene expression in animal models, including nonhuman primates.[12]

Of the 7 patients who were receiving prophylaxis with FIX concentrate before gene transfer, 4 were able to stop regular FIX replacement therapy, and most of the others were able to increase the interval between prophylactic infusions. Despite the reduction in the use of FIX concentrate, the average annual number of bleeding episodes was consistently lower after gene transfer, most notably in patients in the high-dose cohort, for whom the mean steady-state FIX expression was around 5% of the normal value. This reduction in the number of annual bleeding episodes occurred despite higher overall physical activity levels reported by all patients, including participation in sports activities that had previously been associated with bleeding. This improvement in the bleeding phenotype among patients in the high-dose cohort is consistent with the natural bleeding tendency in patients with mild haemophilia who have plasma FIX levels of 5 to 40%. Such patients have either no or very few spontaneous bleeding episodes but are at risk for excessive haemorrhage after trauma or surgery.[13]

The major vector-related adverse event (AE) 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. Expansion of the high-dose cohort showed that this AE was common, occurring in 4 of the 6 patients but was well managed through the administration of prednisolone. The increase in serum ALT levels occurred consistently at 7 to 10 weeks after gene transfer, thus defining the critical period of monitoring and pharmacologic intervention. In other studies using a similar construct (George et al NEJM 2017), the same asymptomatic increase in ALT requiring steroid treatment has been seen as early as 4 weeks post treatment with a dose of 5 x 10^{11} vg/kg.[14]

5.4 Rationale and Risks/Benefits

5.4.1 Rationale

Haemophilia B is an X-linked recessive bleeding disorder that affects approximately 1 in 30,000 males. The disorder results from a defect in the *f9* gene and is characterised by frequent spontaneous bleeding into joints and soft tissues which, without adequate treatment, causes a chronic debilitating arthropathy.

Current treatment for HB in the developed world involves either IV infusions of plasma-derived or recombinant factor concentrates at the time of a bleed (on-demand therapy) or regular infusions of clotting factors to prevent bleeds (prophylactic treatment). On-demand treatment is highly effective at arresting haemorrhage but cannot totally abrogate chronic damage that

ensues after a bleed. In severely affected patients, bleeding episodes can be dramatically reduced by prophylactic treatment when plasma FIX levels are maintained at or above only 1% of normal by regular administration of FIX protein concentrates.[1] The relatively short half-life of FIX necessitates frequent IV administration of factor concentrates (2-3 times a week) which is invasive, inconvenient and problematic for children.

Following administration of AAV gene therapy, the target cells are transduced. AAV gene therapy is poor at integrating into the host genome and the transgene remains episomally in those transduced cells. It is from this position that the transgene is expressed and, in the case of FIX, this is a steady, continuous expression. This continuous synthesis of FIX by the host cells after gene therapy offers a real opportunity to prevent bleeding episodes and eliminating the need for regular infusions. In addition, current experience suggests that the level of efficacy seen with gene therapy is likely to be superior to that seen with prophylactic treatment and, when considering the most recent data, may even offer the prospect of a functional cure and a lack of haemorrhage even in response to trauma or surgery.

This study aims to investigate the safety of a new gene therapy (FLT180a) and investigate its potential to alter the disease phenotype from severe to mild/normal through the endogenous production of FIX following a single administration of vector.

Dose Selection

The objective is to select an initial dose of FLT180a, based on studies in non-human primates and extrapolation from results in previous studies that will result in FIX levels that would, at a minimum, reduce disease severity whilst balancing the risk of toxicity.

In the study conducted by Nathwani et al, IV infusion of AAV2/8-LP1-hFIXco at low (2×10^{11} vector genomes [vg]/kilogram [kg]), intermediate (6×10^{11} vg/kg), or high dose (2×10^{12} vg/kg) resulted in in a 1.5%, 2%, and 5% level of hFIX respectively. Asymptomatic, transient transaminitis occurred in 4 of 6 patients at high dose level (at 7-10 weeks) but was effectively managed by administration of a short course of prednisolone with liver enzymes normalised and hFIX levels retained in the 2-4% range.

The FLT180a (AAV2/S3-[redacted]-Ti-FIXco1) investigational medicinal product (IMP) has been designed with the following potential enhancements which are hypothesised to confer an increase in FIX activity over and above what was achieved with the previous construct:

(1) A new small synthetic liver promoter ([redacted])

(2) An enhanced transgene including

   a. codon optimised FIX gene

   b. a gain-of-function Padua mutation (R338L)

   c. truncated intron in a natural position

(3) A novel engineered capsid (AAV-S3) developed from wild-type AAV3 and AAV8 through capsid shuffling. This enables the vector to transduce human hepatocytes with high efficiency.

In vivo data in mice models are not predictive of transgene expression in humans and therefore the proposed doses have been estimated based on extrapolation of *in vitro* data in human cells comparing the performance of elements of the AAV2/8-LP1-hFIXco construct used in the previous study by Nathwani et al. and those incorporated in FLT180a.[11,15,16,17] Please refer to the investigator brochure for full details.

The planned low cohort dose of FLT180a in this study (low dose: 6×10^{11} vg/kg of body weight) will be in line with the intermediate dose utilised in the study conducted by Nathwani et al. Table 3: FIX Activity Projections illustrates projections for FIX activity in the current study based on putative enhancements in the new construct.

Table 3: FIX Activity Projections

Dose (vg/kg)	FIX levels (% of normal) achieved in published FIX trial (Nathwani et al) (Using AAV2/8-LP1-hFIXco)	FIX levels (% of normal) projected for FLT180a in this study
2×10^{11}	1.5	-
6×10^{11}	2	30
2×10^{12}	5	75
4×10^{12}	Not studied	>75

Abbreviations: FIX = Factor IX; kg = kilogram; vg = vector genomes.

Depending on safety outcome (including AE reporting and laboratory tests) and results of FIX levels, dose cohort escalation may occur provided there is no more than 1 dose-limiting toxicity (DLT) at any dose level and if the resulting FIX activity fails to reach the target range. A DLT is defined as any grade 3 or greater AE at least possibly related to FLT180a. The goal for FIX response is to maximise the number of patients who achieve a FIX activity in the range 70-150% whilst minimising the risk of overshooting the normal physiological range. A FIX activity in the range 70-150% represents normalisation to a level at which patients may undergo surgical procedures without the requirement for additional exogenous factor concentrates.[18] The dose will be escalated in increments to a maximum dose of 4×10^{12} vg/kg (high dose). In order to reduce the risk of overshooting the normal physiological range, reduction of the dose level within a dose cohort may occur if FIX activity exceeds pre-defined levels in the initial patients. Where a dose reduction occurs the 2+1 design will apply at that new dose level within the cohort. Additional patients may be added to a dose cohort to ensure adequate characterisation of any DLT, safety issues not meeting DLT criteria, or the FIX response, at the request of the trial management group and data monitoring committee and at the discretion of the Sponsor (see Section 12).

Two Good Laboratory Practice toxicology studies using FLT180 and FLT180a have been conducted in Rhesus Macaques.

In the first study using FLT180 and FLT180a, animals received doses of 3.34 x 10^{13} vg/kg and 1.235 x 10^{12} vg/kg, respectively. For FLT180, this is 50-fold greater than the starting dose in cohort 1, and approximately 8-fold greater than the dose in cohort 3. For FLT180a this is 2-fold the starting dose in cohort 1. There were no adverse findings in this study during the 8 and 4 week assessment period respectively.

In a supplementary toxicology study conducted with FLT180a at a dose of 3.41 x 10^{13} vg/kg (50-fold greater than the starting dose in cohort 1 and approximately 8 times the maximum human dose proposed) there were adverse histopathological findings in one animal that were likely associated with high hFIX levels (>12-times normal) 8 weeks post dose and considered an exaggerated pharmacological effect, thrombotic effects have been seen in one non-human primate. The occurrence of antibodies to hFIX in this animal may have resulted in an under estimation of the hFIX levels and therefore higher levels of hFIX may have been produced in the animal with the lung lesion.

Peak hFIX levels up to 170% and sustained hFIX levels of 110% of normal have not resulted in FLT180a associated adverse effects and in this context the nonclinical data is considered to support dosing in this study. This protocol mandates rigorous monitoring of FIX levels in patients and includes strict controls around inter patient interval and stopping rules that are designed to minimise risk in the clinic associated with supraphysiological FIX levels.

Dosing and Dose Escalation Interval

In order to monitor for acutely occurring, dose-limiting AEs and to minimise the risk of overshooting the normal physiological range for FIX, a 4-week interval between dosing patients will be enforced during the dose escalation phase to monitor for acutely occurring dose-limiting AEs. This interval can be extended should there be any safety concerns (see Section 12.3.2 and Section 12.3.3).

Analysis of the data from the studies conducted by Nathwani et al[11,17] has shown a marked effect of corticosteroid treatment on FIX activity levels. An increase of 2.5 to 3-fold in FIX levels have been observed in parallel with the prednisolone regimen (unpublished data), these return to pre-steroid levels approximately 5 weeks following cessation. The steroid response with the smaller [redacted] promoter used in FLT180a is expected to be less profound but not absent and therefore pre-steroid FIX levels (week 3) will be used to guide dose escalation decisions.

Based on data from a similar single stranded AAV vector currently in development (George et al NEJM 2017) FIX activity levels at 3 weeks represent approximately 65% of those achieved at plateau.[14] Cohort data review will utilise the 65% FIX level at week 3 to project an expected steady state and will guide dose escalation/reduction decisions.

As this will be a first time in human study for FLT180a, an extended 6-week interval will be observed following dosing of the first patient in the study and prior to dosing the second patient to minimise risk to subsequent patients of unanticipated AEs.

The main AE from previous studies has been an asymptomatic increase in serum ALT level associated with a decline in FIX levels occurring typically at week 7-10 following dosing in the study by Nathwani et al.[11] To date these events have been effectively managed with rapid introduction of a short course of corticosteroids. In other studies using a similar construct (George et al NEJM 2017), the same asymptomatic increase in ALT requiring steroid treatment has been seen as early as 4 weeks post treatment with a dose of 5×10^{11} vg/kg.[14]

All patients will be given a take-home pack of immunosuppressants to be taken under the direction of the investigator, which will allow rapid intervention if transaminase elevations are observed. In addition, during the anticipated critical time period all patients will receive a course of immunosuppressants commencing at the week 3 visit.

As patient follow-up will potentially be carried out across multiple sites, it will be a requirement for all investigators with active patients who have received FLT180a to confirm the absence of safety issues in patients under their care that would prevent further patient dosing/dose escalation. Dosing of subsequent patients will not be allowed until this confirmation is received.

5.4.2 **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 neutralising antibody production, and that such antibodies preclude re-administration of AAV of the same serotype although other serotypes can be successfully re-administered. It is therefore highly probable that patients who receive AAV-S3 whilst participating in this study will develop neutralising antibodies and that any future administration of vector of this serotype would be ineffective. This event becomes more problematic if the individual patient receives a sub-therapeutic dose. The sponsors objective is therefore to select an initial dose, based on studies in non-human primates and extrapolation from results in previous studies that may result in FIX levels that would, at a minimum, reduce disease severity (see section on Dose Selection). Experience from the first-generation FIX study conducted by Nathwani et al demonstrates that activity remains stable (>6 years) but natural turnover of liver cells would suggest that it is possible for levels to decline over time. An initial therapeutic level therefore might become sub-therapeutic in the future increasing the importance of careful evaluation of benefit/risk in dose selection.

A major mitigating factor against this risk is that in both murine and non-human primate models, vector particles of an alternative serotype can be administered with subsequent predictable expression of FIX.[1] Thus, if patients enrol into this trial and receive a subtherapeutic dose, they could be considered for future trials of AAV vectors of an alternate serotype with a potentially therapeutic benefit despite the presence of antibodies to AAV-S3.

Screening for Liver Abnormalities

Patients with evidence of liver dysfunction (persistently elevated ALT, AST, bilirubin >1.5xULN) are excluded from the study. All patients will undergo a liver ultrasound. Based on patient

history and outcome of the ultrasound, additional tests like fibroscan, MRI and elastography may be requested to exclude significant fibrosis prior to inclusion in the study with the input of the Chief Investigator. Results of these tests should be discussed with the Chief Investigator and Sponsor in order to confirm eligibility for the study.

Vector-induced Hepatitis and Loss of FIX Expression

In the study conducted by Nathwani et al, the major vector-related AE was a dose-dependent, asymptomatic increase in the serum ALT level associated with a decline in FIX levels, suggesting a loss of transduced hepatocytes. Expansion of the high-dose cohort showed that this AE was common, occurring in 4 of the 6 patients but was well managed through the administration of prednisolone. The increase in serum ALT levels occurred consistently at 7 to 10 weeks after gene transfer, thus defining the critical period of monitoring and pharmacologic intervention. In other studies using a similar construct (George et al NEJM 2017), the same asymptomatic increase in ALT requiring steroid treatment has been seen as early as 4 weeks post treatment with a dose of 5×10^{11} vg/kg.[14]

Patients will be given a take-home pack of immunosuppressants to be used in the event of transaminase elevation. Patients will be monitored intensively over the course of the study for evidence of immune hepatitis and a short course of immunosuppressants will be mandated for all patients commencing at the week 3 visit. Outside of this time, patients will be instructed not to take these immunosuppressants unless instructed to do so by the investigator however availability of immunosuppressants will facilitate rapid initiation of treatment if necessary.

Immunosuppressants to Suppress Immune Hepatitis

The long-term use of high dose corticosteroids may be associated with the development of side effects. These include high blood pressure, elevation in blood sugar, weight gain with increased appetite and fluid retention. Osteoporosis is generally considered a long-term side effect and other bone complications include 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 irritation may occur and could result in gastrointestinal haemorrhage. Emotional disturbances and mood changes are also described.

For patients entering the study with a history of hepatitis, there is a risk of disease reactivation associated with immune suppression. Monitoring for hepatitis B/C reactivation will be implemented for patients with a history of hepatitis B and C in parallel with the introduction of the immunosuppression regimen.

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, hyperkalaemia, hypertension, hyperglycaemia, insomnia, visual disturbances, headaches, hyperphosphatemia, tremor, myocardial hypertrophy, pure red cell aplasia, haemolytic uremic syndrome, posterior reversible encephalopathy syndrome. Use of live vaccines for immunisation should be avoided.

Patients should also avoid prolonged exposure to UV light and sunlight by wearing protective clothing and sunscreen with a high protection factor (minimum SPF30) and avoid grapefruit juice/ grapefruit during use of tacrolimus.

The risk of side effects to the immunosuppressants will be minimised by careful monitoring, utilisation 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 immune hepatitis begins to subside.

A management guideline for tacrolimus dosing is available in Appendix 3.

Monitoring for CMV reactivation will be implemented for all patients positive for CMV (IgG) at screening in parallel with the introduction of the immunosuppression regimen. In the instance of CMV reactivation a management guideline for CMV reactivation is available in Appendix 2.

Patients with pre-existing contraindications to the immunosuppressants are excluded from the trial.

Transduction of Extra-hepatic Tissues

Following systemic administration of AAV-S3, there is potential for the spread of vector particles to non-hepatic tissues including the gonads. Therefore, shedding will be assessed in samples taken 3 times over the first 10 days of the study. Thereafter samples will be taken weekly until week 12 and then at visits 14, 16, 20 and 26 as required until no further evidence of FLT180a is detectable (as determined by 3 consecutive clear samples). As a precaution, only patients 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 Neutralising Anti-human FIX Antibodies

A significant concern of this study, as with previous studies, is that research subjects may develop an immune response to FIX protein following systemic administration of FLT180a. In macaques, neutralising antibodies have been observed in a small proportion of animals exposed to human GTMP, including FLT180a. Investigations suggest that these antibodies are provoked by expression of a xenoprotein in non-human primates.[1,13] However, several studies indicate that endogenous synthesis of FIX in the liver reduces the risk of developing inhibitors.[8,9,10] To enhance the safety of AAV-mediated FIX expression, a liver-specific regulatory element ([redacted]) is contained within the expression cassette which limits transgene expression to hepatocytes.

All patients eligible for the study will have received >150 exposure days to factor concentrates and are therefore less likely to develop inhibitors following AAV infusion and liver transduction.[19]

Patients who have an inhibitor or a history of inhibitor after protein replacement therapy will be excluded from the study.

Insertional mutagenesis and/or tumorigenesis

Insertional mutagenesis leading to oncogenesis is a recognised 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 potentially rare events.

Deep sequencing studies in murine and nonhuman primate models have shown that integration of the AAV genome can occur in the liver.[20,21,22] Additionally, studies in mice have reported an increased incidence of hepatocellular carcinoma following perinatal or neonatal gene transfer.[23,24] 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 tumourigenesis.[24,25]

Liver biopsies taken from six of nine adult patients (age range: 33-62 years) one year after receiving AAV2/5 hybrid vector (with a liver specific human α-1 antitrypsin promoter), for the treatment of acute intermittent porphyria, have shown a scarce random integration of vector at non-specific locations with no evidence of malignant transformation. Patients with acute of chronic liver disease were excluded from this study.[26,27]

Conversely, AAV infection has been indicated as having a protective role against cancer.[28]

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. Patients will be followed for up to 15 years and will be fully informed regarding risk of mutagenesis prior to study entry.

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 severe HB patients routinely self-administer factor concentrates IV without significant procedure related side effects. Standard procedures that are well established in comprehensive haemophilia treatment centres in Europe, Africa and North America will be followed to ensure the safe and uneventful administration of vector IV and during venepuncture for blood tests. Bleeding in the soft tissues around the venepuncture site will be treated with FIX concentrates, if necessary.

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.[29] Patients should be informed of early symptoms and signs of hypersensitivity reactions, including hives, generalized urticaria, angioedema, chest tightness, dyspnoea, wheezing, faintness, hypotension, tachycardia, and anaphylaxis. Patients will remain under observation in the investigational centre for at least 12 hours following infusion

to minimise 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 infusion and then every 2 hours for 6 hours.

Thrombogenicity

The aim of the protocol is to reduce disease severity in HB patients dosed with FLT180a by increasing FIX expression. This is a first time in man protocol, and FIX expression approximations may have been underestimated.

The expression cassette incorporates the gain-of-function Padua mutation. This is a naturally occurring mutation and originates from an Italian family in which, despite normal levels of FIX antigen, the hemizygous mother had 337% of the normal FIX activity level, a younger son (pre-puberty) 551% of the normal activity level and an older son (23 years) 776% of the normal level of activity.[30] Despite the very high FIX activities seen in this family, there was no family history of venous thrombosis until the older son (FIX activity 776% of normal) presented with a diagnosis of occlusive femoral–popliteal deep-vein thrombosis in the right leg.

In the supplementary toxicology study conducted with FLT180a at a dose of 3.41 x 10^{13} vg/kg (50-fold greater than the starting dose in cohort 1) there were adverse histopathological findings in one animal that were likely associated with high hFIX levels (>1200%) and considered an exaggerated pharmacological effect.

The World Federation of Hemophilia (WFH) regard 150% as the upper limit of normal for Factor IX activity.

Should FIX activity achieved exceed the normal physiological range, there is a risk of thrombogenicity. The risk is considered to be very low based on the magnitude of FIX activity with apparent lack of symptomatology for many years in the family where the Padua mutation was first described and on studies in mice and dogs expressing the Padua mutation. In one study, sustained hFIX expression reached approximately 200% hFIX activity and 30% of antigen levels respectively with no clinical or laboratory evidence of thrombosis.[31]

Patients will however be informed of early symptoms and signs of thrombotic phenomena, including pain and/or tenderness along a vein, swelling of an arm or leg without pain or tenderness, redness along a vein, low fever without any known reason (such as a cold or flu), sudden shortness of breath or difficulty breathing or coughing, sudden chest pain, sudden severe headache or changes in vision, and numbness or tingling in arms or legs. If such an event occurs whilst the patient is at home, the patient will be instructed to seek immediate medical care.

In the instance of supratherapeutic FIX levels guidelines for management and thromboprophylaxis are included in section 12.3.3.

Patients at high risk of thromboembolic events are excluded from the trial.

Myocarditis

Two sets of toxicity studies with a caesium chloride purified vector preparation were performed in mice to support clinical trials with AAV2/8-LP1-hFIXco, the first-generation FIX gene therapy trial that were conducted by Nathwani et al. Animals in the first cohort received vector preparation without modification. These animals experienced both cardiac thrombosis and myocarditis. The second cohort received vector which was 10-fold concentrated. Thrombosis and myocarditis were again seen, but this cohort had pronounced hepatic necrosis. All the mice that exhibited toxicity had hFIX levels greater than 300 µg/mL, which is 60 times the upper limit of normal in humans (FIX activity approximately 6000%). Myocardial toxicity in transgenic mice secondary to high levels of FIX has been described previously.[32] Toxicity in mice was only observed at very high levels of hFIX, therefore these effects are not expected to occur in study participants whose FIX levels are anticipated to remain in the normal range after gene transfer. Cardiac monitoring with electrocardiogram (ECG) is however implemented as part of the scheduled assessments and should be followed by echocardiogram if necessary.

Risks associated with DNA impurities

DNA impurities in AAV vector preparations, derived from production plasmids used for transient transfection, are well described.[33,34,35]

The plasmid system used in manufacture of FLT180a reduces the likelihood of replication-competent AAV (rcAAV) generation compared with previous products. The incidence of rcAAV in FLT180a has been shown to be very low at less than 10 rcAAV in 1 x 10^{10} vector genomes, i.e. below the limit of detection of the validated assay method.

Packaging of prokaryotic DNA impurities, including the kanamycin antibiotic resistance gene, derived from production plasmids has also been detected at levels ranging from 1.2 to 6.3% in AAV vectors generated by transfection of HEK293 cells or by helper-virus infection of stable producer cell lines. Kanamycin sequences were present in the FLT180a GMP batch 17-086 at a level of 0.74% vector genomes. No other batches will be used for this study.

Therefore, the administration to humans of the clinical lot of FLT180a is not considered to result in toxicity due to the presence of DNA impurities. Their role in the initiation of liver transaminitis however cannot be excluded especially as bacterial DNA itself can generate an immune response in humans but we believe that transient immunosuppression with immunosuppressants will suppress liver cytotoxicity and preserve long-term transgene expression from hepatocytes.

Unknown Risks Associated With First Time in Human ATIMP

This is a first time in human clinical trial of an advanced therapy and as such there may be as yet uncharacterised risks that have not been evident in preclinical species. A comprehensive battery of safety assessments is included in the protocol but in order to mitigate against as yet unknown risks, the following additional steps will be taken.

The first 2 patients at each dose level will remain as inpatients at the infusion centre for 24 hours following dosing. All infusion centres will have access to emergency facilities.

An extended 6-week interval will be observed following dosing of the first patient in the study and prior to dosing the second patient to minimise risk to subsequent patients of unanticipated delayed AEs.

5.4.3 Assessment and Management of Risk

This trial is categorised 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.

6 Objectives

6.1 Primary

Safety

To assess the safety of systemic administration of FLT180a in adults with HB at up to 3 different dose cohorts.

Efficacy

To assess FIX levels following systemic administration of FLT180a, at the terminal dose level.

6.2 Secondary

- To investigate the endogenous production of FIX following 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 and exogenous FIX consumption.
- To assess the immune response to the FIX transgene product following systemic administration of FLT180a.
- To assess viral shedding in various body fluids after systemic administration of FLT180a.

6.3 Exploratory

- To assess the immune response to the AAV-S3 capsid proteins following systemic administration of FLT180a.

Following a single administration of FLT180a:

- To investigate the impact of endogenous production of FIX on functional status and disability in HB.
- To investigate the impact of endogenous production of FIX on quality of life (QoL) in HB.
- To investigate the impact of endogenous production of FIX on physical activity in HB.
- To investigate the impact of endogenous production of FIX on haemophilia health status in HB.
- To investigate the impact of endogenous production of FIX on joint health in HB.

- To investigate the impact of endogenous production of FIX on health resource utilisation in HB.

7 Trial design

7.1 Overall Design

This is a phase I/II, open label, multicentre, ascending single dose, safety study of FLT180a in patients with severe HB.

Patients will undergo the screening assessments described in Section 11.1.1 and the Schedule of Assessments (Table 1: Schedule of Assessments) up to 52 weeks prior to Study Day 0 (gene therapy infusion). Due to the risk of bleeding in this patient group, a washout from the patients FIX concentrate regimen is not mandated as part of this protocol. The investigator must however be able to demonstrate, from the patients' medical records, a documented FIX activity level of <1% for severe patients or <2% for moderately severe patients. If (at the investigators discretion) a FIX concentrate washout is undertaken during the screening period, a minimum of 5 days washout is required.

Treatment-eligible patients will report to the study site on the day prior to receiving the gene therapy infusion (Day -1, NB. Day -1 assessments may be conducted as early as Day -3 for logistical reasons). On Day 0, FLT180a will be administered as a single dose, slow IV infusion into a peripheral vein, and the patient will remain in the study centre for at least 12 hours and until the investigator has deemed the patient as fit to be discharged. The first 2 patients treated at each dose level will remain at the study centre for 24 hours following infusion prior to discharge.

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

Patients will be required to undergo study evaluations at intervals over the 26-week post treatment period. These will take place either at the study infusion site or at their normal haemophilia treatment centre. In order to monitor for shedding of vector genome sequences, patients will be required to provide plasma, saliva, urine, stool and semen samples until 3 successive samples are shown to be clear.

This is a first-in-human trial and as such an ascending-dose design has been implemented to enable dose evaluation in a step-wise manner. 3 dose cohorts of vector (low, intermediate, and high) will be tested in the dose escalation. Two patients will be tested at each dose level with an additional patient added in the event of a DLT (2 + 1 design). Dose escalation may occur provided there is no more than 1 DLT at any dose cohort and if the resulting FIX activity fails to reach the target level. A reduction of the dose level within a cohort may occur if FIX

activity exceeds defined levels in order to reduce the risk of overshooting the normal physiological range. Where a dose reduction occurs the 2 + 1 design will apply at that new dose level within the cohort. At the discretion of the Sponsor following advice from the trial management group (TMG) and independent data monitoring committee (DMC), additional patients may be added to any cohort to ensure adequate characterisation of either safety or the FIX response prior to dose escalation/reductions. The Sponsor, trial management group and data monitoring committee will select the terminal dose level based on the patient FIX activity levels with the aim of ensuring the majority of patients will reach a FIX activity within normal limits and in the absence of dose-limiting AEs, the terminal dose level will be expanded to 14 patients. This design minimises the number of patients 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. An extended 6-week interval will be observed between the first and second patient on study to monitor for any unanticipated delayed AEs. Subsequently, whilst dose escalation is ongoing, the study mandates a minimum 4-week interval between patients during which time efficacy and safety will be reviewed prior to a decision to dose the next patient. Once the terminal dose level is agreed and cohort expansion initiated, the dosing interval between patients will be reduced to 48 hours.

The main risk in this study is considered to be a dose-dependent, asymptomatic increase in the serum ALT level associated with a decline in FIX levels, suggesting a loss of transduced hepatocytes. In the study conducted by Nathwani et al, transaminase elevations tended to occur between weeks 7-10 and were effectively managed by a short course of corticosteroids. In the study by George et al (NEJM 2017) the same asymptomatic increase in ALT requiring steroid treatment has been seen as early as 4 weeks post treatment with a dose of 5×10^{11} vg/kg.[14] In this study, all patients will be given a take-home pack of immunosuppressants following infusion and transaminase levels will be monitored closely. During the critical time period, from the week 3 visit, all patients will receive immunosuppressants, at all other times patients will be instructed to start the course of immunosuppressants only if told to do so by the investigator.

The main efficacy endpoint will be based on an analysis of the proportion of patients achieving a clinical or normalised FIX response at 26 weeks. A clinical FIX response is defined as achieving a FIX activity of 5% to 150% of normal. Five percent has been selected as the threshold for a clinical FIX response because using gene therapy in haemophilia B patients to increase FIX activity from <1% to 5% has been shown to lead to a highly clinically significant improvement in annualised bleed rates and exogenous factor consumption.[11,17] A normalised FIX response is defined as achieving a FIX activity level in the normal range (50-150% - per the World Federation of Haemophilia).[36] The normal range has been selected as the threshold level for a normalised FIX response because reaching this level will modify the patient phenotype from severe at study start to normal at which point patients would not experience spontaneous bleeds.

The choice of a 26-week endpoint is based on previous experience with AAV gene therapy for HB (Nathwani et al) in which patients achieved steady-state FIX levels by 16 weeks post gene

therapy. Based on this, it is anticipated that the patients FIX activity will have reached a stable level by 26 weeks, thus this is an appropriate point at which to measure activity.

7.2 Endpoints

7.2.1 Primary Endpoints

Safety

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

Efficacy

The following primary endpoints will be analysed:

- The proportion of patients achieving clinical FIX response at Week 26, at the terminal dose level. A clinical FIX response is 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 is defined as achieving FIX activity in the normal range (50-150%).[38]

7.2.2 Secondary Endpoints

Safety

Safety as assessed by reporting of 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 annualised bleeding rate.
- Change from baseline in FIX concentrate consumption.

In order 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 will be from day 15 inclusive.

Immune Response

Immune response to the FIX transgene product (i.e., development of inhibitors) will be assessed by measurement of the level of inhibitors.

Shedding

Clearance of vg in plasma, saliva, urine, stool, and semen.

7.2.3 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 will be 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 EQ-5D-5L and the Haem-A-QoL score.

Haemophilia Health Status

Change from baseline in the 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.

8 Selection of Patients

Patient enrolment at a site will only commence once the trial has:

- documented Ethics Committee, Competent Authority and Local Institution approval.
- been initiated on behalf of the sponsor.
- been issued with a site activation letter on behalf of the sponsor.

Patients may only be enrolled at approved trial sites.

8.1 Inclusion Criteria

1. Adults 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. Able to give full informed consent and able to comply with all requirements of the trial including 15-year long-term follow-up.
4. Willing to practice barrier contraception until at least 3 consecutive semen samples after vector administration are negative for vector sequences.
5. Lack of neutralising anti-AAV-S3 antibodies using an *in vivo* transduction inhibition assay within 4 weeks of vector administration.
6. At least 150 exposure days to FIX concentrates.

8.2 Exclusion 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 would include those with a history of arterial or venous thromboembolism (e.g. deep vein thrombosis, pulmonary embolism, non-haemorrhagic stroke, arterial embolus) and those with acquired thrombophilia including conditions such as atrial fibrilation).
3. Use of investigational therapy for haemophilia within 30 days before enrolment.

4. Patients with active hepatitis B or C, and HBsAg or hepatitis C virus (HCV) RNA viral load positivity, respectively, or currently on antiviral therapy for hepatitis B or C. Negative viral assays in 2 samples, collected at least 6 months apart, will be required to be considered negative. Both natural clearers and those who have cleared HCV on antiviral therapy are eligible.
5. Serological evidence of HIV-1.
6. Evidence of liver dysfunction (persistently elevated alanine aminotransferase, aspartate aminotransferase, bilirubin >1.5 x upper limit of normal).
7. Platelet count <50 x 10^9/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. Prior treatment with any gene transfer medicinal product.
13. Known or suspected intolerance, hypersensitivity or contrainidication to the investigational product and non-investigational medicinal products or their excipients.
14. Planned major elective surgery prior to the end of trial.
15. Current or relavant history of a physical or psychiatric illness or any medicial condition that in the opinion of the investigator could affect the patients safety or interfere with the study assessments.
16. CMV IgG postive patients who are CMV PCR positive at screening.

9 Investigational Medicinal Products and Non-Investigational Medicinal Products

9.1 Name and Description of ATIMP(s) and IMP(s)

FLT180a (AAV2/S3-[redacted]-Ti-FIXco1) is a replication-incompetent single-stranded recombinant AAV vector. The vector is composed of a single-stranded DNA genome encapsidated in an AAV-derived protein capsid. FLT180a possesses 3 novel features:

(1) A new small synthetic liver promoter ([redacted])

(2) An enhanced transgene including

   a. codon optimised FIX gene

   b. a gain-of-function Padua mutation (R338L)

   c. truncated intron in a natural position

(3) A novel engineered capsid (AAV-S3) developed from wild-type AAV3 and AAV8 through capsid shuffling. This enables the vector to transduce human hepatocytes with high efficiency.

FLT180a is formulated [redacted] The drug product is presented as a sterile, aqueous concentrate for solution for infusion, supplied in 10 mL vials. Each vial contains 5 mL of FLT180a Concentrate for Solution for Infusion. The vials are sealed with rubber stoppers and aluminium seals with plastic flip tops.

FLT180a Concentrate for Solution for Infusion is stored at <-60°C and must remain frozen until thawed prior to use.

FLT180a is classified as an advanced therapy investigational medicinal product (ATIMP) and specifically a gene therapy medicinal product.

9.2 Source of ATIMP/IMP, Manufacture, Distribution and Storage

9.2.1 Manufacture

FLT180a Concentrate for Solution for Infusion is manufactured according to Good Manufacturing Practice (GMP) by:

Children's GMP LLC
St. Jude Children's Research Hospital
Memphis, Tennessee
USA

An audit of Children's GMP LLC has been performed by a Qualified Person (QP) from the Clinical Biotechnology Centre who are responsible for importation into the UK and QP certification (Section 9.2.3).

9.2.2 Manufacture and Shipment

A clinical batch of FLT180a Concentrate for Solution for Infusion will be manufactured according to GMP and tested according to a product specification. The genomic titre will be determined as part of the product release testing and will be stated on the Certificate of Analysis and included on the product labelling. The genomic titre stated on the labelling and Certificate of Analysis should be used for all dose calculations.

Investigational medicinal product will be labelled in accordance with local regulatory requirements.

Shipment of the IMP will be undertaken by a company specialising in cold chain transportation of biopharmaceuticals. Vials will be maintained at <-60°C during shipment. All shipments will include temperature loggers so that the temperature control during transit can be confirmed.

9.2.3 Importation into the UK, QP Certification and Storage Prior to Shipment to Investigational Site

Importation of the IMP into the UK will be performed by:

Clinical Biotechnology Centre (CBC)
University of Bristol
Langford House
Lower Langford
Bristol
BS40 5DU

Qualified Person (QP) certification of the batch will be performed by an appropriately experienced QP from CBC. The batch manufacturing record, test results and the certificate of analysis for the clinical batch will be provided to the QP for the purposes of batch certification.

In the event that IMP cannot be certified by the QP, it will be retained under quarantine at CBC.

CBC will also perform secondary packaging and labelling in the UK, including labelling with expiry date. Investigational medicinal product will be stored at CBC at <-60^{o}C until shipped to the investigational sites for administration.

9.2.4 Shipment of IMP to Infusion Sites

FLT180a Concentrate for Solution for Infusion will be shipped from CBC to the UK infusion site. The appropriate number of vials required for dosing will be determined based on the weight of each patient. The vials will be shipped to the infusion site prior to the scheduled dosing day by a company specialising in cold chain transportation of biopharmaceuticals. All shipments will contain temperature monitoring devices.

FLT180a Concentrate for Solution for Infusion required for dosing in the US will be retained at the site of manufacturing which is also the infusion site. No shipment is therefore required.

9.2.5 Infusion Sites

Administration of IMP will be restricted to centralised infusion sites.

Royal Free Hospital
Pond Street
Hampstead
London NW3 2QG

St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
USA

9.2.6 Receipt Storage and Handling of ATIMP(s)/IMP(s) at Site

All IMP aspects of the trial at participating sites are the responsibility of the Principal Investigator (PI), 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.

FLT180a will be received at the investigational site by the PI or designee. The vials will be inspected to ensure that they have remained frozen in transit and will be transferred to a restricted access freezer maintaining a temperature of <-60^{o}C.

Once predose eligibility checks have been confirmed on Day 0, the vector will be thawed and diluted. All procedures will be performed in a standard biological safety cabinet which has been cleaned by each site's standard cleaning procedure.

Detailed instructions on receipt, storage and handling of FLT180a Concentrate for Solution for Infusion are contained in the ATIMP management plan. Instructions for the preparation of FLT180a infusion solutions are also included in the ATIMP management plan. All local requirements related to activities involving genetically modified organisms will be adhered to.

9.2.7 Accountability and Traceability of ATIMP(s)/IMP(s)

There is a system set-up to ensure the traceability of each ATIMP from the starting material, through to administration to the participant and destruction or final transfer. A comprehensive ATIMP management plan 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 trial site(s) to retain their part of the traceability information is set out in the relevant contractual agreements with the Sponsor.

9.3 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 non-investigational medicinal product (NIMP) in this trial and will be sourced from stocks at the infusion centre or local haemophilia treatment centre.

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

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

9.4 Implementation of the Immunosuppressants Regimen

Patients will be instructed to only take treatment as instructed by the investigator.

[redacted]

If break through transaminitis occurs, follow the instructions below.

Break through transaminitis

- When a patient has an elevation in ALT beyond the upper limit of normal (ULN) or the trends of LFT and/or Factor IX levels suggest potential transaminitis or a lack of response to the immunosuppression regimen:
    - Evaluate to exclude other causes of transaminitis.
    - Assume it is vector induced transaminitis after reasonable attempts to exclude other causes. Record/report the event accordingly in the source documentation and eCRF.
    - Contact the Chief Investigator immediately.
    - Administer methylprednisolone 1g IV for 3 days;

      Check tacrolimus levels and if required adjust dose to ensure therapeutic levels. If tacrolimus is not currently in use, initiate immediately. Management guidelines for tacrolimus dosing are contained within Appendix 3.

Following methylprednisolone give prednisolone at 1mg/kg/day.

- Monitor LFTs daily initially to establish response to treatment by evaluating the trend in ALT levels. Once a response to treatment has been established continue prednisolone 1mg/kg/day with regular monitoring of the LFTs, two or 3 times a week, until the LFTs are within normal range and continue for an additional week and then initiate prednisolone taper as described above. Tacrolimus can be discontinued at any time in consultation with the Chief Investigator and Medical Monitor.

- The Investigator, in consultation with the Chief Investigator and Medical Monitor, 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.

At all times, Investigators should be vigilant of a downward trend in FIX activity level, which alongside an elevation in ALT, may be an early indicator of transaminitis.

Where there is contact or consultation with the Chief Investigator, the Sponsor should be kept informed.

10 Concomitant Medication

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

10.1 Prior Treatment

Prior treatment includes all treatment received from 30 days prior to the initial informed consent up until the time of FLT180a infusion. Prior treatment information must be recorded on the appropriate eCRF page.

10.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the date of investigational product infusion and the week 26/end of study visit, inclusive. All concomitant treatments information must be recorded on the appropriate eCRF page.

Patients 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.

Other therapy considered necessary for the patient's welfare may be given at the discretion of the investigator. All such therapy must be recorded in the eCRF. No other IMP may be used

concomitantly with the study treatment. The patients are not allowed to participate concurrently in another clinical study.

10.3 Withdrawal From Existing FIX Concentrate Therapy

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

11 Study Procedures

11.1 Study Schedule

11.1.1 Screening

The following trial-specific procedures will be carried out after the initial informed consent form (ICF) is signed to assess the participant's eligibility. Screening evaluations may be performed at the infusion study centre or in the patient's haemophilia treatment centre. A window of up to 52 weeks (from initial consent to day -1) is allowed for screening to allow for controlled prospective collection of bleeding event and FIX consumption data via the patient diary. A patient can move forward to Day -1 assessments and dosing as dosing slots are available. There is no minimum time frame for the data collection. ALL screening assessments should be completed in a timely manner (~2 weeks) following consent to confirm patient eligibility and may take place across multiple visits to complete all assessments. During screening the patient should be contacted ~every 4 weeks to check on diary completion and collect details on any AEs. A repeat AAV Antibody screen is mandated within 4 weeks of dosing. If the screening period for a patient is longer than 16 weeks then a repeat of the central laboratory screening bloods (FIX Antigen/ HBV, HCV, HIV, CMV Screen/ Haematology, Chemistry incl. CRP, Coagulation Screen/ Liver Function Test/ FIX Activity Level/ FIX Inhibitor Level) will be required within 4 weeks of dosing and the patient reported outcomes should also be repeated at Day -1. Patients may be rescreened should this time window elapse.

- Demographics will be recorded.
- Medical history will be documented.
- WHO performance status will be documented.
- Prior and concomitant medication will be documented.
- A bleeding and FIX concentrate utilisation history will be documented (3-year history) and any target joints will be recorded.
- A physical examination will be conducted including measurement of the patient's height and weight. Waist circumference, hip circumference, neck circumference and bioimpedance will also be measured.

- A 12-lead ECG will be conducted.
- Vital signs (pulse, blood pressure) will be measured.
- A clinical assessment of joints will be undertaken using the HJHS.
- A liver ultrasound scan will be conducted.
- AAV-S3 antibody screening will be conducted. Note: A blood sample drawn within 4 weeks of dosing must be confirmed as negative before a patient can be considered for dosing. It is acceptable to take 2 samples during the screening period. Should there be any problems with the testing it is acceptable to take repeat samples as necessary.
- Blood samples will be taken for HIV, HCV (anti-HCV antibodies and HCV RNA viral load, HCV RNA viral load only indicated for patients with history of HCV and positive HCV antibody test), HBV, CMV (CMV IgG test and CMV PCR; CMV PCR only indicated for patients with positive CMV IgG) screening. Note: a repeat blood sample drawn within 4 weeks of dosing for testing will required if the patient is in the screening period for longer than 16 weeks.
- Blood samples will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (central lab). Note: a repeat blood sample drawn within 4 weeks of dosing for testing will required if the patient is in the screening period for longer than 16 weeks.
- FIX activity level (trough).
  - For patients on on-demand therapy, a blood sample should be taken following 5 days free from factor concentrates.
  - For patients on conventional prophylactic therapy, a blood sample should be taken at the end of the longest acceptable interval between doses.
  - For patients taking EHL prophylactic therapy, a blood sample should be taken within the 48-hour period before the next planned dose.
- Baseline FIX activity (following 5 days washout or obtained from documented medical records).
- A blood sample will be taken to evaluate FIX inhibitor level. Note: a repeat blood sample drawn within 4 weeks of dosing for testing will required if the patient is in the screening period for longer than 16 weeks.
- A blood sample will be taken to evaluate FIX antigen level. Note: a repeat blood sample drawn within 4 weeks of dosing for testing will required if the patient is in the screening period for longer than 16 weeks.
- FIX genotype will be recorded from the patients' medical notes. (if not available this test may be conducted as part of the trial)
- Quality of life will be evaluated (EQ-5D-5L and Haem-A-QoL).
- Disability status will be assessed (WHODAS 2.0).

- Physical activity status will be assessed (HAL 2005).
- Haemophilia health status will be assessed (PROBE).
- Health resource utilisation will be documented (6-month history).
- A bleeding/FIX consumption diary will be issued to the patient with instructions on completion.
- Adverse events will be recorded during the screening period from the time of initial consent.

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

11.1.2 **Vector Infusion (Day -1 to Day +4)**

Vector infusion will only occur once all screening assessments have been conducted and the patient is deemed to be eligible for the study. Eligible patients will attend a qualified infusion centre prior to the day of vector infusion and an additional 'pre-infusion' dosing site informed consent will be obtained from each patient. On the day prior to vector infusion, local safety laboratory tests will be utilised for management of patients to ensure rapid turnaround of clinical results. Duplicate safety blood samples will be drawn at day -1 to provide a baseline for both central and local laboratories. For all consenting patients the following procedures will be undertaken:

<u>Day -1</u>

- A second informed consent will be taken – this may occur prior to Day -1.
- A physical examination will be conducted (including confirmation of weight). Waist circumference, hip circumference, neck circumference and bioimpedance will also be measured.
- Vital signs (pulse, blood pressure, respiration rate, and temperature) will be measured.
- A 12-lead ECG will be conducted.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab). For list of required tests please see Table 2: Detailed Schedule of Assessments for Infusion Week and Section 11.4.3.2.
- Blood samples will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (central lab).
- Blood will be taken for evaluation of FIX activity level (sample for both local and central lab).

- Blood will be taken for collection of peripheral blood mononuclear cells (Elispot and Research). The mononuclear (Research) sample is optional.
- A blood sample will be taken and stored. This sample may be used to retrospectively analyse AAV-S3 antibody status immediately prior to dosing.
- PCR of vg in plasma, saliva, urine, stool, and semen.
- Adverse event and concomitant medication status will be recorded.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.
- Plasma samples will be drawn and frozen for research purposes. These samples are optional.
- Plasma samples will be drawn and frozen for immune response research purposes.

If the screening period for a patient is longer than 16 weeks then the following will also be completed on Day -1:

- Quality of life will be evaluated (EQ-5D-5L and Haem-A-QoL).
- Disability status will be assessed (WHODAS 2.0).
- Physical activity status will be assessed (HAL 2005).
- Haemophilia health status will be assessed (PROBE).
- Health resource utilisation will be documented (since screening).

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 ATIMP management plan.
- 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 patient's vital signs and blood samples will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab). For list of required tests please see Table 2: Detailed Schedule of Assessments for Infusion Week and Section 11.4.3.2. Blood will also be taken for evaluation of FIX activity level (sample for both local and central lab).
- The calculated FLT180a vector dose will be infused over 1 hour through the catheter using an appropriate infusion pump.
- Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored at 15 minute intervals (+/-5 mins) from the start to completion of infusion.
- On completion of the infusion, the catheter will again be flushed with saline.

- Vital signs will be monitored hourly for 6 hours (+/- 10 mins) from the end of infusion and then every 2 hours (+/-15 mins) for a further 6 hours.
- Laboratory safety blood samples, haematology, chemistry incl. CRP, coagulation screen and LFTs will be repeated at 8 hours after the end of the infusion. For list of required tests please see Table 2: Detailed Schedule of Assessments for Infusion Week and Section 11.4.3.2.
- Adverse event and concomitant medication status will be recorded.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.
- Patients will remain hospitalised for 12 hours to observe for any immediate toxicity of the procedure. If the vital signs are stable, the catheter will be removed prior to patient discharge from the centre.
- The first 2 patients at each dose level will remain at the study centre for 24 hours following infusion with additional vital signs monitoring at 16 and 20 hours (+/-15 mins) following infusion.
- All patients will be discharged with a medical alert card.

Day +1, +2, and +4

During the first week following infusion, evaluations will be performed exclusively at the study centre where the participant received their vector infusion by the investigator or designee.

At each visit:

- A physical examination will be conducted.
- Vital signs (pulse, blood pressure, respiration rate, and temperature) will be measured.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab). For list of required tests please see Table 2: Detailed Schedule of Assessments for Infusion Week and Section 11.4.3.2.
- Blood will be taken for haematology, chemistry incl. CRP and coagulation screen (central lab) on day 2 and 4.
- Blood will be taken for evaluation of FIX activity level on day 2 and 4 (local lab).
- Adverse event and concomitant medication status will be recorded.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.
- Plasma, saliva, urine, stool and semen samples will be taken for polymerase chain reaction (PCR) of vg on day 2 and 4.
- In addition, on Day +1, a 12-lead ECG will be conducted.

11.2 Follow-up Visits (Weeks 1-25)

After the first week, evaluations may be performed in the patient's regular haemophilia treatment centre or at the patient's home (in the case of routine blood sampling and vital signs monitoring).

11.2.1 Week 1-5 Assessments

The following assessments will be conducted at the **main** visit each week (or as specified):

- A physical examination will be conducted.
- Vital signs (pulse, blood pressure) will be measured.
- A 12-lead ECG will be conducted at week 2 and 4.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (central lab).
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab).
- Blood will be taken for collection of mononuclear cells for immune studies (Elispot).
- Blood will be taken for evaluation of FIX activity levels (central and local lab).
- Blood will be taken for evaluation of FIX inhibitor levels (weeks 1, 2, 3).
- Commencing at the week 3 visit, patients will receive a course of immunosuppressants (see Section 9.4).
- For any patients with a history of HBV and HCV, local testing for hepatitis B or C reactivation should be conducted at week 4.
- From week 3 CMV testing (CMV PCR; in patients with CMV positive (IgG) at screening) should be conducted weekly in conjunction with immunosuppressant dosing. Management guidelines in the case of CMV reactivation can be found in Appendix 2.
- From week 3 tacrolimus levels should be measured at each blood draw until therapeutic levels are established and weekly thereafter in conjunction with immunosuppressant dosing. Management guidelines for tacrolimus dosing can be found in Appendix 3.
- Blood will be taken for evaluation of AAV-S3 antibody titre (weeks 1, 2).
- Plasma, saliva, urine, stool, and semen samples will be taken for PCR of vg (three times within 7-10 days of vector infusion and weekly thereafter). These collections will be done unless there have been 3 prior consecutive specimens that were negative.
- Adverse event and concomitant medication status will be recorded at each visit.
- Plasma samples will be drawn and frozen for research purposes. These samples are optional.

- Plasma samples will be drawn and frozen for FIX activity assay research purposes (weeks 1 and 4).
- Plasma samples will be drawn and frozen for immune response research purposes only during the course of investigations or treatment of breakthrough transaminitis.

Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 2 further separate occasions each week during week 1-5 (see Section 11.2.10).

11.2.2 **Week 6-12 Assessments**

The following assessments will be conducted at the **main** visit each week (or as specified):

- A physical examination will be conducted.
- Vital signs (pulse, blood pressure) will be measured.
- A 12-lead ECG will be conducted at week 8.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (central lab).
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab).
- Blood will be taken for collection of mononuclear cells for immune studies (Elispot).
- Blood will be taken for evaluation of FIX activity levels (central and local lab).
- Blood will be taken for evaluation of FIX antigen levels (week 12).
- Blood will be taken for evaluation of FIX inhibitor levels (weeks 6, 9, 12).
- Patients will continue to receive a course of immunosuppressants (see Section 9.4).
- For any patients with a history of HBV and HCV, local testing for hepatitis B or C reactivation should be conducted at week 6, 8, 10, 12.
- CMV testing (CMV PCR; in patients with CMV positive (IgG) at screening) should be conducted weekly in conjunction with immunosuppressant dosing. Management guidelines in the case of CMV reactivation can be found in Appendix 2.
- From week 3 tacrolimus levels should be measured at each blood draw until therapeutic levels are established and weekly thereafter in conjunction with immunosuppressant dosing. Management guidelines for tacrolimus dosing can be found in Appendix 3.
- Blood will be taken for evaluation of AAV-S3 antibody titre (week 6, 12).
- Plasma, saliva, urine, stool, and semen samples will be taken for PCR of vg weekly. These collections will be done unless there have been 3 prior consecutive specimens that were negative.
- Adverse event and concomitant medication status will be recorded at each visit.

- Plasma samples will be drawn and frozen for research purposes. These samples are optional.
- Plasma samples will be drawn and frozen for immune response research purposes only during the course of investigations or treatment of breakthrough transaminitis.

Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 1 further separate occasion each week during week 6-12 (see Section 11.2.10).

11.2.3 **Week 13 Assessments**

- Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 2 separate occasions during week 13 (see Section 11.2.10). Blood draws may be taken either at study site or at alternative location (e.g. patient's home). The intention should be for at least one of the two blood draws per week to be conducted at study site.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab), once in week 13. Blood draw may be taken either at study site or at alternative location (e.g. patient's home).
- CMV testing (CMV PCR; in patients with CMV positive (IgG) at screening) should be conducted in conjunction with immunosuppressant dosing, as required.
- Tacrolimus level should be conducted in conjunction with immunosuppressant dosing, as required.
- Adverse event and concomitant medication status will be recorded and ongoing diary completion assessed.

11.2.4 **Week 14 Assessments**

The following assessments will be conducted at the **main** visit:

- A physical examination will be conducted.
- Vital signs (pulse, blood pressure) will be measured.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (central lab).
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab).
- Blood will be taken for collection of mononuclear cells for immune studies (Elispot).
- Blood will be taken for evaluation of FIX activity levels (central and local lab).
- Patients will continue to receive a course of immunosuppressants (see Section 9.4).
- For any patients with a history of HBV and HCV, local testing for hepatitis B or C reactivation should be conducted.

- CMV testing (CMV PCR; in patients with CMV positive (IgG) at screening) should be conducted in conjunction with immunosuppressant dosing. Management guidelines in the case of CMV reactivation can be found in Appendix 2.
- Tacrolimus levels should be measured in conjunction with immunosuppressant dosing. Management guidelines for tacrolimus dosing can be found in Appendix 3.
- Plasma, saliva, urine, stool, and semen samples will be taken for PCR of vg. These collections will be done unless there have been 3 prior consecutive specimens that were negative.
- Adverse event and concomitant medication status will be recorded at each visit.
- Plasma samples will be drawn and frozen for research purposes. These samples are optional.
- Plasma samples will be drawn and frozen for immune response research purposes only during the course of investigations or treatment of breakthrough transaminitis.

Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 1 further separate occasion during week 14 (see Section 11.2.10).

11.2.5 **Week 15 Assessments**

- Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 2 separate occasions during week 15 (see Section 11.2.10). Blood draws may be taken either at study site or at alternative location (e.g. patient's home). The intention should be for at least one of the two blood draws per week to be conducted at study site.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab), once in week 15. Blood draw may be taken either at study site or at alternative location (e.g. patient's home).
- CMV testing (CMV PCR; in patients with CMV positive (IgG) at screening) should be conducted in conjunction with immunosuppressant dosing, as required.
- Tacrolimus level should be conducted in conjunction with immunosuppressant dosing, as required.
- Adverse event and concomitant medication status will be recorded and ongoing diary completion assessed.

11.2.6 **Week 16 Assessments**

The following assessments will be conducted at the **main** visit:

- A physical examination will be conducted.
- Vital signs (pulse, blood pressure) will be measured.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.

- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (central lab).
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab).
- Blood will be taken for collection of mononuclear cells for immune studies (Elispot).
- Blood will be taken for evaluation of FIX activity levels (central and local lab).
- Blood will be taken for evaluation of FIX inhibitor levels.
- Patients will continue to receive a course of immunosuppressants (see Section 9.4).
- For any patients with a history of HBV and HCV, local testing for hepatitis B or C reactivation should be conducted.
- CMV testing (CMV PCR; in patients with CMV positive (IgG) at screening) should be conducted in conjunction with immunosuppressant dosing. Management guidelines in the case of CMV reactivation can be found in Appendix 2.
- Tacrolimus levels should be measured in conjunction with immunosuppressant dosing. Management guidelines for tacrolimus dosing can be found in Appendix 3.
- Plasma, saliva, urine, stool, and semen samples will be taken for PCR of vg. These collections will be done unless there have been 3 prior consecutive specimens that were negative.
- Adverse event and concomitant medication status will be recorded at each visit.
- Plasma samples will be drawn and frozen for research purposes. These samples are optional.
- Plasma samples will be drawn and frozen for immune response research purposes only during the course of investigations or treatment of breakthrough transaminitis.

Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 1 further separate occasion during week 16 (see Section 11.2.10).

11.2.7 **Week 17-19 Assessments**

- Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 2 separate occasions each week during week 17-19 (see Section 11.2.10). Blood draws may be taken either at study site or at alternative location (e.g. patient's home). The intention should be for at least one of the two blood draws per week to be conducted at study site.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab), once a week, weeks 17-19. Blood draw may be taken either at study site or at alternative location (e.g. patient's home).
- For any patients with a history of HBV and HCV, local testing for hepatitis B or C reactivation should be conducted at week 18.

- CMV testing (CMV PCR; in patients with CMV positive (IgG) at screening) should be conducted in conjunction with immunosuppressant dosing, as required.
- Tacrolimus level should be conducted in conjunction with immunosuppressant dosing, as required.
- Adverse event and concomitant medication status will be recorded and ongoing diary completion assessed.

11.2.8 **Week 20 Assessments**

The following assessments will be conducted at the **main** visit:

- A physical examination will be conducted.
- Vital signs (pulse, blood pressure) will be measured.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (central lab).
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab).
- Blood will be taken for collection of mononuclear cells for immune studies (Elispot).
- Blood will be taken for evaluation of FIX activity levels (central and local lab).
- Blood will be taken for evaluation of FIX inhibitor levels.
- Patients will discontinue the course of immunosuppressants (see Section 9.4).
- For any patients with a history of HBV and HCV, local testing for hepatitis B or C reactivation should be conducted.
- CMV testing (CMV PCR; in patients with CMV positive (IgG) at screening) should be conducted in conjunction with immunosuppressant dosing. Management guidelines in the case of CMV reactivation can be found in Appendix 2.
- Tacrolimus levels should be measured in conjunction with immunosuppressant dosing. Management guidelines for tacrolimus dosing can be found in Appendix 3.
- Plasma, saliva, urine, stool, and semen samples will be taken for PCR of vg. These collections will be done unless there have been 3 prior consecutive specimens that were negative.
- Adverse event and concomitant medication status will be recorded at each visit.
- Plasma samples will be drawn and frozen for research purposes. These samples are optional.
- Plasma samples will be drawn and frozen for immune response research purposes only during the course of investigations or treatment of breakthrough transaminitis.

Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 1 further separate occasion during week 20 (see Section 11.2.10).

11.2.9 **Week 21-25 Assessments**

- Frequent local laboratory assessments (FIX activity level and LFTs) will be conducted on 2 separate occasions each week during week 21-25 (see Section 11.2.10). Blood draws may be taken either at study site or at alternative location (e.g. patient's home). The intention should be for at least one of the two blood draws per week to be conducted at study site.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab), once a week, weeks 21-25. Blood draw may be taken either at study site or at alternative location (e.g. patient's home).
- Adverse event and concomitant medication status will be recorded and ongoing diary completion assessed.

11.2.10 **Frequent Local Laboratory Assessments**

In addition to the main weekly visit schedule, the following laboratory assessments will be conducted an additional 2 times per week (3 times in total) between week 1-5 and an additional 1 time per week (2 times in total) between week 6-12, week 14, week 16 and week 20. For week 13, 15, 17, 18, 19, 21, 22, 23, 24 and 25 the following laboratory assessments will be conducted 2 times per week. The tests should be as evenly spaced through the week as possible, for example: 3 times per week – Monday, Wednesday and Friday; 2 times per week – Monday and Thursday or Tuesday and Friday. These samples may be taken at study site or at an alternative location (e.g. patient's home) by a home nursing vendor. For week 13, 15, 17, 18, 19, 21, 22, 23, 24 and 25 the intention should be for at least one of the two blood draws per week to be conducted at study site. To facilitate rapid evaluation of results and intervention where necessary, local laboratory analysis should be used for assessments during this period:

- FIX activity level.
- LFTs - for list of required tests please see Table 1: Schedule of Assessments and Section 11.4.3.2.

In response to an upward trend in ALT, frequency of LFT and FIX activity level assessments should be increased in line with the guidance in Section 9.4.

Where required to meet the requested schedule of testing these frequent assessments should include sample for reactivation of hepatitis, tacrolimus levels and CMV testing as per Table 1: Scheduled of Assessments. These samples may be taken at study site or at an alternative location (e.g. patient's home) by a home nursing vendor.

11.3 Week 26/End-of-Study Visit

The following assessments will be conducted at week 26. Should the patient discontinue the study at an earlier time point, every attempt should be made to conduct the week 26 assessments to ensure adequate safety follow-up.

- A physical examination will be conducted.
- Vital signs (pulse, blood pressure) will be measured.
- A 12-lead ECG will be conducted.
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (central lab).
- Blood will be taken for haematology, chemistry incl. CRP, coagulation screen and LFTs (local lab).
- Blood will be taken for collection of mononuclear cells for immune studies (Elispot and Research). The mononuclear (Research) sample is optional
- Blood will be taken for evaluation of FIX activity levels (central and local lab).
- Blood will be taken for evaluation of FIX antigen levels.
- Blood will be taken for evaluation of FIX inhibitor levels.
- Blood will be taken for evaluation of AAV-S3 antibody titre.
- Plasma, saliva, urine, stool, and semen samples will be taken for PCR of vg. These collections will be done unless there have been 3 prior consecutive specimens that were negative.
- Plasma samples will be drawn and frozen for research purposes. These samples are optional.
- Plasma samples will be drawn and frozen for FIX activity assay research purposes.
- Plasma samples will be drawn and frozen for immune response research purposes.
- Adverse event and concomitant medication status will be recorded.
- Assessment of bleeding episodes and FIX usage based on patient diaries and direct questioning.
- A clinical assessment of joints will be undertaken using the HJHS.
- A liver ultrasound scan will be conducted.
- Quality of life will be evaluated (EQ-5D-5L and Haem-A-QoL).
- Disability status will be assessed (WHODAS 2.0).
- Physical activity status will be assessed (HAL 2005).
- Haemophilia health status will be assessed (PROBE).
- Health resource utilisation will be evaluated since screening or Day -1.

11.4 Study Procedures/Evaluations

11.4.1 Demographic and Baseline Assessments

11.4.1.1 Informed Consent Procedure

The informed consent process will be conducted twice during the study. Initially at screening and then again once eligibility has been confirmed but prior to FLT180a infusion.

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

A mandatory meeting with an independent patient advocate, who will be tasked with focusing on the risks associated with the trial, will be arranged prior to the second 'pre-infusion' dosing site informed consent to ensure that the participant fully comprehends the risks. The right to withdraw at any time during the study without prejudice will also be explained again. The requirement for a meeting with an independent patient advocate will not apply once the terminal dose level is selected.

A copy of the signed ICF will be given to the participant. 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, patients will be informed that in the event of death, no matter what cause, permission for an autopsy will be requested of their families. Patients will be asked to advise their families of this request and of its scientific and medical importance should they choose to participate.

11.4.1.2 Patient Identification Code

On enrolment to the study, the patient will be given a unique patient 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 patient number (e.g., 004). Using these specifications, the fourth patient enrolled at site 03 in the United Kingdom (country code 44) will have the unique patient identification code 44031004. Allocation of the patient identification code will be handled through the eCRF.

11.4.1.3 Factor IX Mutation Genotype

Factor IX mutation genotype should be transcribed onto the eCRF from the patients' medical records during the screening period. Where genotype has not been established, a blood sample will be sent to the central laboratory for analysis.

11.4.1.4 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.

11.4.1.5 Bleeding History

The investigator should document the patients bleeding and FIX utilisation history over the preceding 3 years.

From this data target joint assessment will be recorded as per the criteria below[38]:

A target joint is defined as one in which there have been three or more spontaneous bleeds within a consecutive 6-month period. Where there have been ≤2 bleeds into the joint within a consecutive 12-month period the joint is no longer considered a target joint.

11.4.1.6 AAV-S3 Neutralising Antibody Screening

During the screening period, a blood sample will be taken from the patient to determine the presence or absence of neutralising antibodies to the AAV-S3 serotype.

A negative result from a transduction inhibition assay within 4 weeks of dosing is required to confirm eligibility. It is acceptable to take 2 samples during the screening period. Should there be any problems with the testing it is acceptable to take repeat samples as necessary. At each time-point a primary sample will be drawn and plasma sent for analysis. A secondary sample will be drawn and plasma frozen locally as a back-up sample.

Procedures for collection, processing, storing, and transporting of samples to the central laboratory are fully described in the study Laboratory Manual.

11.4.1.7 HIV, HCV, HBV, CMV Screening

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

- Hepatitis B virus surface antigen (HBsAg).
- Anti-hepatitis C virus (anti-HCV) antibodies and Hepatitis C RNA viral load. HCV RNA viral load only indicated for patients with history of HCV and positive HCV antibody test.
- Human immunodeficiency virus 1 and 2 (anti-HIV1/2) antibodies.
- Cytomegalovirus IgG antibodies and Cytomegalovirus PCR. CMV PCR only indicated if patients are positive on CMV IgG.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.2 **Efficacy**

11.4.2.1 Baseline FIX Activity

Baseline FIX activity must be established for all patients.

Due to the risk of bleeding in this patient group, a washout from the patients FIX concentrate regimen is not mandated as part of this protocol. The investigator must however be able to demonstrate a documented FIX activity level of <1% for severe patients or <2% for moderately severe patients from the patients' historical medical records.

For patients where a washout is undertaken as part of this protocol, a minimum of 5 days off therapy is required. In this case the FIX activity trough sample will also be used as the baseline value.

11.4.2.2 FIX Activity Trough

A trough level of FIX activity should be established during the screening period.

- For patients on on-demand therapy, a blood sample should be taken following 5 days free from factor concentrates.
- For patients on conventional prophylactic therapy, a blood sample should be taken at the end of the longest acceptable interval between doses.
- For patients taking EHL prophylactic therapy, a blood sample should be taken within the 48-hour period before the next planned dose.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.2.3 FIX Activity

Blood samples for assessment of FIX activity will be drawn in accordance with Table 1: Schedule of Assessments and Table 2: Detailed Schedule of Assessments for Infusion Week. Samples at the various time-points will be analysed at a central laboratory (efficacy) and local laboratory (efficacy and safety) as indicated.

Timepoints for a central laboratory sample:

A primary sample will be drawn and plasma sent for analysis of FIX activity.

A secondary sample will be drawn and plasma frozen. The secondary sample will be used to establish FIX activity in an alternative investigational assays.

Procedures for collection, processing, storing, and transporting to the laboratory are fully described in the study Laboratory Manual.

Timepoints for a local laboratory sample:

Local blood samples will be taken Day -1, Day 0 (pre-dose), Day +2, Day +4, 3 times per week between weeks 1-5, 2 times per week between weeks 6-25 and once in week 26/EOS. These samples will be analysed locally. 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).

Local laboratory analysis will be utilised to ensure rapid turnaround of results in this exception.

11.4.2.4 FIX Antigen

Blood samples for assessment of FIX antigen will be drawn in accordance with Table 1: Schedule of Assessments.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.2.5 Bleeding Episodes

Bleeding episodes will be entered into the patient's diary and will include:

- Number
- Location
- Aetiology (spontaneous, traumatic, etc)
- Total units of FIX concentrate required to resolve the bleed

Diary data will be reviewed with the investigator at the next available visit.

11.4.2.6 Factor IX Concentrate Usage

For each dose of FIX concentrate administered the patient will record the concentrate, dose given in total international unit (IU), the reason for administration (prevention, bleeding episode), and the frequency of administration in the patient diary.

Diary data will be reviewed with the investigator at the next available visit.

11.4.3 **Safety**

11.4.3.1 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 patient'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.

11.4.3.2 Laboratory Safety Assessments

Blood samples for safety assessments will be drawn in accordance with Table 1: Schedule of Assessments and Table 2: Detailed Schedule of Assessments for Infusion Week.

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

- Haematology: complete blood count with differential, platelet count.
- Chemistry incl. CRP: sodium, potassium, calcium, chloride, phosphate, CO_2, glucose, blood urea nitrogen, serum creatinine, C-reactive protein.
- Coagulation screen: prothrombin time, activated partial thromboplastin time, prothrombin split fragments 1+2 (F1+2), D-dimer, TAT.
- Liver Function Tests: albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase, aspartate aminotransferase, total protein, GGT.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

The following laboratory tests will be performed at a local laboratory to assess safety.

- Haematology: complete blood count with differential, platelet count.
- Chemistry incl. CRP: sodium, potassium, phosphate, blood urea nitrogen or urea, serum creatinine (and estimated GFR), C-reactive protein.
- Coagulation screen: prothrombin time, activated partial thromboplastin time.
- Liver Function Tests: albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase, aspartate aminotransferase.
- FIX activity level.

Full local laboratory safety blood samples will be taken Day -1, Day 0 (pre-dose and +8 hours), Day +1, Day +2, Day +4. Samples for LFT's and FIX activity level will be taken 3 times per week between weeks 1-5, 2 times per week between weeks 6-25 and once at week 26/EOS. These samples will be analysed locally.

Local laboratory analysis will be utilised to ensure rapid turnaround of results in this exception.

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).

Blood samples for reactivation of hepatitis, tacrolimus levels and CMV testing will be drawn in accordance with Table 1: Schedule of Assessments and Section 9.4 and analysed locally. 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 in the case of CMV reactivation can be found in Appendix 2. Management guidelines for tacrolimus dosing can be found in Appendix 3.

If immunosuppressants are implemented at any other time, monitoring for reactivation of hepatitis B/C, tacrolimus levels and CMV testing should be undertaken in conjunction with the immunosuppressant dosing.

11.4.3.3 FIX Inhibitor

Blood samples for assessment of FIX neutralising antibody development (inhibitor) will be drawn in accordance with Table 1: Schedule of Assessments.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.3.4 AAV-S3 Antibody Titre

Blood samples for assessment of AAV-S3 Antibodies will be drawn in accordance with Table 1: Schedule of Assessments. A primary sample will be drawn and plasma sent for analysis. A secondary sample will be drawn and plasma frozen locally as a back-up sample.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.3.5 Vital Signs

Measurements of vital signs (blood pressure, pulse, temperature, and respiratory rate) will be performed according to the study schedule (Table 1: Schedule of Assessments and Table 2:

Detailed Schedule of Assessments for Infusion Week). 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.

11.4.3.6 ECG

A 12-lead ECG will be conducted in accordance with the study schedule (Table 1: Schedule of Assessments and Table 2: Detailed Schedule of Assessments for Infusion Week).

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

11.4.3.7 Liver Ultrasound

A liver ultrasound will be conducted in accordance with the study schedule (Table 1: Schedule of Assessments).

Any clinically significant deviations from screening should be reported as an AE.

11.4.3.8 Adverse Event Collection

Patients 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., patient diaries, questionnaires, clinical evaluations) to ascertain whether an AE has occurred. Adverse events are collected from the time initial informed consent is signed until week 26. Please refer to Recording and Reporting of Adverse Events in Section 13.

11.4.4 **Vector Shedding**

Plasma, saliva, urine, stool and semen samples for PCR of vector genome will be taken in accordance with Table 1: Schedule of Assessments and Table 2: Detailed Schedule of Assessments for Infusion Week. These collections will occur until there have been 3 consecutive samples that are negative.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.5 **Health Economic Assessments**

Assessments will be conducted in accordance with the study schedule (Table 1: Schedule of Assessments and Table 2: Detailed Schedule of Assessments for Infusion Week).

11.4.5.1 Quality of Life

Quality of life will be evaluated using the EQ-5D-5L and Haem-A-QoL.

11.4.5.2 Disability

Disability will be assessed using the WHODAS 2.0.

11.4.5.3 Physical Activity

Physical activity will be assessed using the HAL 2005.

11.4.5.4 Haemophilia Health Status

Haemophilia health status will be assessed using PROBE.

11.4.5.5 Health Resource Utilisation

The following items will be recorded in the eCRF:

- Number of haemophilia related medical appointments and medical activities
- Number of emergency room visits
- Number of hospitalisations related to haemophilia
- Length of hospital stay
- Number of physiotherapy sessions, specialist consultations and appointments with professional caregivers.

The following items will be captured in the patient diary:

Number of days lost from education or work by patients and caregivers due to bleeding episodes.

11.4.6 **Exploratory**

11.4.6.1 Joint Health/Function

Joint health/function will be conducted in accordance with the study schedule (Table 1: Schedule of Assessments).

Joint health/function will be assessed using the HJHS score.

11.4.6.2 Mononuclear Cells (Immune Studies)

Blood samples will be collected for immune studies in accordance with Table 1: Schedule of Assessments and Table 2: Detailed Schedule of Assessments for Infusion Week.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.6.3 Mononuclear Cells (Research)

At Day -1 and week 26 a second mononuclear cell sample will be taken for research purposes. These samples are optional. Research samples will be retained beyond the end of the study and may be analysed alongside samples taken as part of the long-term follow-up study or pooled with other studies using similar constructs to generate a larger sample for analysis. Any analysis will be conduct independent of the study report. The samples may therefore be held for up to 15 years and analysed as part of future research which has been ethically approved.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.6.4 Research Plasma

Research plasma samples will be collected in accordance with Table 1: Schedule of Assessments and Table 2: Detailed Schedule of Assessments for Infusion Week for research

purposes. These samples are optional. Research samples will be retained beyond the end of the study and may be analysed alongside samples taken as part of the long-term follow-up study or pooled with other studies using similar constructs to generate a larger sample for analysis. Any analysis will be conduct independent of the study report. The samples may therefore be held for up to 15 years and analysed as part of future research which has been ethically approved.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.6.5 FIX Activity Research Plasma

Blood samples will be collected for FIX activity research studies in accordance with Table 1: Schedule of Assessments. The intent of drawing these samples is to investigate the variability of the FIX activity assay in accordance with the draft FDA guidance for gene therapy in haemophilia.[39]

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.4.6.6 Immune Response Research Plasma

Blood samples will be collected for immune response research studies in response to elevations in LFT's and in accordance with Table 1: Schedule of Assessments and Table 2: Detailed Schedule of Assessments for Infusion Week. The intent of drawing these samples is to investigate immune response biomarkers at time-points when events of breakthrough transaminitis are observed.

Procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

11.5 Volume of Blood to be Drawn From Each Patient

Table 4: Volume of Blood to be Drawn From Each Patient for Central Laboratory Testing

Assessment		**Sample Volume (mL)**	**Number of Samples Taken**	**Total Volume (mL)**
AAV-S3 antibody screen/titre		4.5	7	31.5
Genotyping		2	1	2
Safety	Chemistry (including CRP and LFTs where combined and serology - HBV,	3.5 or 5 (where serology included)	20 of which 1 serology	71.5

Table 4: Volume of Blood to be Drawn From Each Patient for Central Laboratory Testing				
Assessment		**Sample Volume (mL)**	**Number of Samples Taken**	**Total Volume (mL)**
	HCV, HIV, CMV as required)			
	HCV RNA viral load	5	1	5
	CMV PCR	5	1	5
	Haematology	2	20	40
	Coagulation	4.5	20	90
FIX activity		9	20	180
FIX inhibitor		4.5	10	45
FIX antigen		4.5	3	13.5
Viral shedding		2	5 (min) to 19 (max)	10-38
Mononuclear Cells (Elispot)		20	17	340
Mononuclear Cells (Research)		20	2	40
Research Plasma Sample		4.5	17	76.5
FIX Activity Research Plasma Sample		20	3	60
Immune Response Research Plasma Sample		4.5	2 (min) to 17 (max)	4.5-76.5
Total mL (Approximate)				1015-1135

Abbreviations: AAV = adeno-associated virus; CMV = cytomegalovirus; CRP = C-reactive protein; FIX = Factor IX; HBV = hepatitis B virus; HCV = hepatitis C virus, HIV = human immunodeficiency virus, max = maximum, min = minimum.

During this study, it is expected that approximately 1015-1135mL of blood will be drawn from all patients for central laboratory assessments.

The protocol allows for monitoring of certain parameters through local laboratories. Blood volume requirements are likely to vary between centres, however, it is estimated that

approximately 1000mL will be drawn 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.

11.6 Discontinuation/Withdrawal of Participants

The reason for discontinuation/withdrawal must be determined by the investigator and recorded in the patient's medical record and on the eCRF. If a patient 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 on the eCRF.

Reasons for discontinuation include but are not limited to:

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

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

In the event of early withdrawal/discontinuation, every effort should be made to collect data in line with week 26/end-of-study assessments.

11.6.1 Withdrawal of Consent to Data Collection

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

11.6.2 Loss to Follow-up

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

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

11.7 Definition of End of Trial

The end of the trial will be defined as the last visit by the last participant.

12 Dose Progression, Dose Escalation and Stopping Rules

The sponsor will retain final responsibility for decision making on all aspects of dose progression, dose escalation/reduction and stopping rules. Progression of dosing within a cohort, dose escalation/reduction and stopping rules are detailed in a study specific plan. The following elements will be included.

12.1 Oversight Committees

12.1.1 Trial Management Group (TMG)

The Trial Management Group (TMG) will include the chief investigator and trial staff, PIs, and sponsor personnel. The TMG will be responsible for overseeing the trial to ensure that the protocol is adhered to and will take appropriate action to safeguard participants and the quality of the trial itself. The group will meet at least monthly and will send updates to PIs who are not in attendance.

The TMG will review trial progress, substantial amendments, patient eligibility and ongoing oversight of patient enrolment within each dose level.

Written approval from the TMG will be mandatory prior to dosing of each patient.

12.1.2 Independent Data Monitoring Committee (DMC)

The role of the DMC is to provide independent advice on data and safety aspects of the trial. Meetings of the committee will be held to review patient data prior to a dose escalation decision, in the case a DLT occurs, selection of the terminal dose or any other issue or safety concern the TMG feel they need advice on from the DMC (i.e. dose reduction). The DMC is advisory to the sponsor and can recommend premature closure of the trial. The DMC will be governed by a charter that dictates constitution and decision making on dose escalation and trial stopping rules.

A positive recommendation from the DMC will be mandatory prior to dose escalation and selection of the terminal dose cohort.

12.1.3 Trial Steering Committee (TSC)

The role of the Trial Steering Committee (TSC) is to provide overall supervision of the trial. The TSC will review the recommendations of the independent DMC and, on consideration of this information, recommend any appropriate amendments/actions for the trial as necessary. The TSC acts on behalf of the funder and Sponsor. The TSC is governed by a charter which dictates constitution and terms of reference.

12.2 Dose-Limiting Toxicity

A DLT is defined as any grade 3 or greater AE at least possibly related to FLT180a except for increases in ALT or AST that are not associated with increases in bilirubin.

Any occurrence of a DLT should be reported as an SAE.

The DMC will review any occurrence of a DLT and further dosing will be suspended until the DMC provides a recommendation to continue.

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

12.3 Dose Escalation

12.3.1 Dose Interval Between Patients

FLT180a will be given as a single dose, slow IV infusion.

The planned dose escalation scheme is as follows:

- Cohort 1 (low dose): 6 x 10^{11} vg/kg of body weight
- Cohort 2 (intermediate dose): 2 x 10^{12} vg/kg
- Cohort 3 (high dose): 4 x 10^{12} vg/kg.

The dose level within a cohort may be reduced based on observed FIX activity levels, see Section 12.3.3.

- Cohort 2 (intermediate dose): 2 x 10^{12} vg/kg can be reduced to:
  - 1.5 x 10^{12} vg/kg
  - 1.3 x 10^{12} vg/kg
  - 1 x 10^{12} vg/kg
  - 8 x 10^{11} vg/kg
- Cohort 3 (high dose): 4 x 10^{12} vg/kg can be reduced to:
  - 3 x 10^{12} vg/kg

As this is a first-in-human study, an extended 6-week interval will be observed between the first and second patient to monitor for any unanticipated delayed AEs.

When applying the rules below, safety will be assessed first (see Section 12.3.2) followed by evaluation of FIX activity (see Section 12.3.3).

During the dose escalation phase of the trial, review of FIX data at a dose of 1.3 x10^{12} vg/kg, is suggestive of the impact of body weight on expression levels. A dose capping at 90 kg is being introduced at this dose (1.3 x10^{12} vg/kg) to ensure that patients FIX activity levels, at steady state are ideally in the target range.

12.3.2 Rules Applicable to Evaluation of Safety in Each Dose Cohort

Dose first patient:

If no DLT is observed, evaluate FIX activity before progressing to dose the second patient.

If DLT arises, then consult DMC prior to dosing second patient and plan for a third patient at the dose level.

Dose second patient:

If no DLT has occurred in either patient at the dose level, evaluate FIX activity before dose escalation (see Section 12.3.3).

If DLT is observed in second patient (with no DLT observed in first patient), consult DMC and plan for addition of third patient at same dose.

If DLT is observed in both patients 1 and 2, apply temporary halt to trial and consult competent authority.

Dose third patient (if necessary):

If no DLT occurs, evaluate FIX activity before dose escalation (see Section 12.3.3).

If DLT observed in third patient and this is second patient in which a DLT has been observed, apply temporary halt to trial and consult competent authority.

The TMG will review safety data for each patient prior to further dosing in line with the rules above. The TMG may recommend enrolment of additional patients be added to any cohort to characterise any safety concern that has not met DLT criteria. Where consulted the DMC must review data and provide recommendation to TMG and Sponsor before another patient is dosed.

Should additional patients be added and 2 DLTs occur at any given dose level then apply temporary halt to trial and consult competent authority.

12.3.3 **Rules Applicable to Assessment of FIX Activity in Each Dose Cohort**

Factor IX activity is expected to follow a pattern similar to that observed with other single-stranded 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 approximately 75% of peak at 4 weeks and achieving steady-state levels at approximately week 12. During steroid administration (from week 3) FIX levels may be exaggerated. As such, week 3 FIX levels (prior to steroid administration) will be used to guide decision making on progression between dose levels.

The target range for FIX activity level at steady state is 70-150%

The TMG will review FIX activity data for each patient prior to further dosing.

The DMC will review FIX activity data for each patient prior to dose escalation, selection of the terminal dose and where requested by the TMG.

The TMG and DMC are in place to protect patient safety and trial validity and will review all data, and projections of anticipated FIX trajectory can be considered, in order to provide recommendations to the Sponsor for dose escalation, terminal dose level selection and dose reductions.

The following rules should be observed to assess dosing decisions in relation to FIX activity and anticipated trajectory:

- FIX activity levels up to and including week 3 (~65% of steady state) will be used to guide decision making for progression between dose levels. The following FIX activity level ranges will be applied and the oversight committees involved in decision making are indicated in brackets:
    - < 50% – dose escalate (TMG and DMC)
    - ≥ 50% and < 60% – consider dose escalation (TMG and DMC)
    - ≥ 60% and < 105% – continue at that dose level (TMG)
    - ≥ 105% and < 150% – TMG evaluates the risk benefit of the dose reduction and recommends the dose for the next patient in keeping with the dose cohorts (TMG and DMC)
    - > 150% – stop dosing further patients until FIX activity levels have peaked*. TMG and DMC will convene to discuss next steps e.g. finding alternate dosing strategies.

    * The attainment of peak FIX activity levels will be judged by the TMG but will generally require evidence over a two-week period showing stability or evidence of continued decline.
- If any patient exceeds 400% at any time stop dosing further patients until FIX activity levels have peaked. TMG and DMC will convene to discuss next steps.
- If any patient FIX levels exceed 200%, consider thromboprophylaxis after individual risk assessment in conjunction with the Chief Investigator. Patients with known risk factors (obesity, smoker, heart failure, older than 60 years age etc.) should be offered thromboprophylaxis (e.g. apixaban) if FIX levels exceed 200%. Patients with no risk factors should be offered thromboprophylaxis if FIX levels exceed 300%. Patients should be closely monitored by the Investigator for signs and symptoms of potential thrombosis. Patients on thromboprophylaxis need to be carefully observed for signs of bleeding. While on thromboprophylaxis, ongoing individual risk assessment of the patient's status including review of FIX levels should be conducted in conjunction with the Chief Investigator. If the risk assessment is favourable and FIX levels return below threshold levels, consideration should be given to stopping thromboprophylaxis.
- If any patient exhibits a positive inhibitor result at any time stop dosing further patients until confirmation of presence of an inhibitor, or not, from a repeat test(s) (see section 12.5 Stopping Rules). TMG and DMC will convene to discuss next steps.
- A minimum of 2 patients treated at any given dose level will be required for dose escalation and selection of terminal dose level decisions.
- Dose reduction decisions (where necessary) can be implemented based on data from 1 patient treated at any given dose level.
- The TMG and DMC may recommend enrolment of additional patients at any dose to further characterise safety or FIX activity level.

- For selection of the terminal dose level, the TMG and DMC will aim to select a dose that enable the majority of patients to attain a FIX activity level in the range 70-150% at steady state. The TMG and DMC will utilise all safety and efficacy data available at the time of decision making. Projection data of FIX activity level from week 4 can be considered where necessary. As such selection of the terminal dose level would be discussed when 2 patients are in the range 50-150% at week 3.
- Once the terminal dose level is selected, 14 patients in total will be dosed at this level. Patients may be dosed at 48-hour intervals. If any patient exceeds 400% at any time stop dosing further patients until FIX activity levels have peaked (see above for definition of peak). TMG and DMC will convene to discuss next steps.
- The TMG and/or DMC will have latitude in the recommendation they make from the start point for discussion and would utilise all safety and efficacy data currently available and projection of anticipated FIX activity at steady state at the dose.

Where more than 2 patients have been dosed at a dose under review it would be expected that the range where the majority of patients FIX activity level falls should be the start point for discussions on the recommendation.

12.4 Temporary Halt

Once a temporary halt is applied to the trial (via submission of a substantial amendment) an internal safety review by the DMC will take place. If the Sponsor deems it appropriate to restart the trial; this can be done only following approval by the relevant competent authorities, ECs and along with any local approvals required.

12.5 Stopping Rules

Further enrolment into the study will be put on hold in the event of any of the following and a substantial amendment will be submitted to temporarily halt the trial:

- Death of a patient related to FLT180a
- Development of malignancy related to FLT180a
- Development of an inhibitor (Anti-FIX antibody) – a positive result must be confirmed by the result of a second sample taken within 1-4 weeks after the initial sample, exhibiting inhibitor, using the Bethesda assay.

Participants 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 trial; this can only be done following approval by all relevant competent authorities, ECs and along with any local approvals required.

13 Recording and Reporting of Adverse Events

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this

treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not related to the medicinal investigational product (International Conference for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guidance E2A 1995).

All AEs are collected from the time the initial ICF is signed until completion of the follow-up period. This includes events occurring during the screening phase of the study, regardless of whether or not investigational product is administered. 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. All AEs should be captured on the appropriate AE pages in the eCRF and in source documents. In addition to untoward AEs, unexpected benefits outside the investigational product indication should also be captured on the AE eCRF.

All AEs must be followed to conclusion regardless of whether the subject is still participating in the study.

Bleeding events in this patient population are not considered as AEs. Bleeding episodes that meet a serious criterion however must be reported as an SAE. All bleeding episodes will be captured in the patient diary throughout the study period.

13.1 Definitions

Term	Definition
Adverse event (AE)	Any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment.
Adverse reaction (AR)	Any untoward and unintended response to an IMP which is **related** to any dose administered to that patient.
Serious adverse event (SAE), serious adverse reaction (SAR) or unexpected serious adverse reaction	Any AE, adverse reaction or unexpected AR, respectively, that: • results in death, • is life-threatening*, • requires hospitalisation** or prolongation of existing hospitalisation, • results in persistent or significant disability or incapacity, or • consists of a congenital anomaly or birth defect.
Unexpected adverse reaction	An AR, the nature and severity of which is not consistent with the information about the medicinal product in question set out: (a) in the case of a product with a marketing authorisation, in the summary of product characteristics for that product,

	(b) in the case of any other IMP, in the investigator's brochure relating to the trial in question.
Suspected unexpected serious adverse reaction (SUSAR)	An unexpected AR which is also categorised as serious.
Important medical event	These events may jeopardise the patient or may require an intervention to prevent one of the above characteristics/consequences. Such events should also be considered "serious."
* A life- threatening event refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. ** Hospitalisation is defined as an in-patient admission, regardless of length of stay. Hospitalisation for pre-existing conditions, including elective procedures do not constitute an SAE.	

13.2 Assessments of Adverse Events

Each AE will be assessed for the following criteria:

13.2.1 Severity

The medical assessment of severity will be determined by using the National Cancer Institute (NCI) CTCAE version 5.0 regardless of causality at each patient assessment. For AEs that are not captured in the NCI CTCAE, the intensity 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

13.2.2 Causality

The investigator must make the assessment of relationship to investigational product for each AE. The investigator should decide whether there is a reasonable possibility that the event may have been caused by the investigational product. 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 investigational

product and the occurrence of the AE, then the AE should be considered "related." The causality assessment must be documented in the source document.

13.2.3 Expectedness

Category	Definition
Expected	An adverse reaction that is classed in nature as serious and which is consistent with the information about the IMP listed in the Investigator Brochure or clearly defined in this protocol.
Unexpected	An adverse reaction that is classed in nature as serious and which is not consistent with the information about the IMP listed in the Investigator Brochure*.

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

The reference document to be used to assess expectedness against the ATIMP is the Investigator Brochure Section 6.11 (Reference Safety Information).

13.2.4 Seriousness

Any AE that meets the criteria for seriousness (see Section 13.1) will be recorded as such within the eCRF.

13.2.5 Serious Adverse Event Reporting Procedures

All initial SAE reports must be reported by the investigator to the CRO pharmacovigilance department within 24 hours of the first awareness of the event, applicable fax numbers and e-mail addresses can be found on the trial specific SAE form. All follow-up reports must be submitted within 24 hours of additional information becoming available. The investigator must complete the SAE Form and transmit the form to the CRO pharmacovigilance department (see Section 1).

13.2.6 Serious Adverse Event Collection Timeframe

All SAEs (regardless of relationship to study) are collected from the time the patient signs the initial ICF until week 26 or end of study. All SAEs must be followed to conclusion regardless of whether the patient is still participating in the study.

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

13.2.7 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 hospitalisations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the patient after signing the ICF, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

13.2.8 **Fatal Outcome**

Any SAE that results in the patient'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 patient's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the patient's death or any ongoing events at the time of death, the action taken with the investigational product should be recorded as "dose not changed" or "not applicable" (if the patient never received investigational product).

13.2.9 **Important Medical Events**

For the purpose of this study the following will be considered as important medical events and will be reported in line with SAE reporting procedures.

- Vector-induced hepatitis (transaminase elevations related to FLT180a).
- Development of an inhibitor.
- A patient develops a grade 2 or greater allergic reaction related to administration of FLT180a.
- Thrombosis.

Other events may be reported at the discretion of the Investigator.

13.2.10 **Pregnancy**

The population under study is male; therefore, pregnancies are not applicable.

A prerequisite to entry into the trial is a willingness to practice a reliable barrier method of contraception until 3 negative semen samples have been obtained after vector administration. In the previous study conducted by Nathwani et al this occurred within 6 weeks in all participants. However, if the partner of a male participant becomes pregnant, before 3 negative semen specimens have been obtained from the patient, the site team should request for permission to keep in touch with their partner to follow-up the pregnancy to its conclusion.

Consent to report information regarding the pregnancy must be obtained from the participant's pregnant partner. A trial-specific pregnancy monitoring information sheet and informed consent form for partners of trial participants must be used for this purpose.

The pregnancy should be reported on a pregnancy reporting form following consent and submitted to the CRO pharmacovigilance department.

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

13.2.11 **Regulatory Agency, Ethics Committee, and Site Reporting**

The CRO is responsible for notifying the relevant regulatory authorities and Central Ethics Committees (ECs)/Institutional Review Boards (IRBs) 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 CRO learning of them with any additional relevant information reported within a further 8 days. Other SUSARs must be reported within 15 days from the CRO learning of them. The CRO will also inform all participating sites.

The investigator is responsible for notifying the local ECs/IRBs or the relevant local regulatory authority of all SAEs that occur at his or her site as required.

The CRO will submit an annual development safety update report to relevant regulatory authorities.

13.2.12 **Expedited Reporting of Other Safety Events**

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

This includes:

- SAEs which could be associated with the trial procedures and which could require modification of the conduct of the trial.
- 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 EC and competent authority by the CRO within 7 days of the CRO learning of them. Events falling into one of the other categories of serious (see Section 13.1 for definition) will be reported by the CRO to the EC and CA within 15 days after the CRO has learned of them.

Expedited reporting and reporting of adverse events will be in accordance with EudraLex Volume 10, Chapter II (Detailed Guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use (‘CT-3’; 2011/C 172/01), June 2011) in the EU and with 21 CFR Part 312 to the FDA.

Should an event also be considered as urgent safety measures, it must be reported in line with the requirements set out below.

13.2.13 **Reporting Urgent Safety Measures**

Urgent safety measures can be put in place with immediate effect without needing to gain prior authorisation by the EC (and competent authority where applicable), in order to protect clinical trial participants from any immediate hazard to their health and safety.

Implementation of urgent safety measures should be notified immediately to the sponsor and CRO who will be responsible for onward reporting to the EC and competent authority.

14 Statistical Considerations

14.1 Statistical Analysis Plan

All study data will be listed and all relevant data will be tabulated and summarised by dose level and overall where appropriate. A Statistical Analysis Plan (SAP) will be written and finalised 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 9.3 or later).

14.2 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 original statistical plan will be captured in the clinical study report.

14.3 Number of Patients

The number of patients enrolled in the dose-escalation phase I/II study will depend on the number of DLTs observed in each cohort and the number of dose levels evaluated. The study will include a minimum of 14 and a maximum of 24 patients. A total of 14 patients will be enrolled at the selected terminal dose level.

14.3.1 Choice of Sample Size

Because of the limited number of patients in the HB population (an estimated incidence of 1 in 30,000 male births), the sample size of this study is based on pragmatic rather than statistical considerations.

The 2 + 1 dose escalation aims to minimise the number of patients 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.

14.4 Demographic and Baseline Characteristics

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

Medical history will be summarised using number of observations and percentages of patients reporting each category.

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

14.5 Study Populations

14.5.1 Analysis Population

The Full Analysis Set will include all patients who received FLT180a. This will be the primary population for all analyses of safety, efficacy and baseline characteristics.

14.5.2 The Per-protocol Population

The Per-protocol Population will include all patients from the Full Analysis Set, excluding those patients with major protocol deviations (following the process described in section 14.11). This data will be used for sensitivity analyses.

The Screened Set will include all patients screened. This set will be used for the listing and summaries of patient disposition and protocol deviations.

14.6 Primary Endpoint

14.6.1 Safety

Adverse events will be coded using CTCAE version 5.0. Frequency of treatment-emergent AEs will be calculated for each body system and preferred term, and by dose level, for number of events and number of patients reporting the event. The severity of the TEAEs and the relationship to study medication will be summarised for each body system and preferred term by dose level.

Withdrawals from the study will be summarised by dose level.

Narratives will be presented for all deaths and patients reporting SAEs.

Toxicities will be graded according to the NCI CTCAE version 5.0.

14.6.2 Efficacy

The FIX response will be derived for each patient based on the FIX activity level measured at the central laboratory. Clinical FIX response is defined as achieving FIX activity of 5-150% and normalised FIX response is defined as achieving FIX activity in the normal range (50-150%).

The two primary endpoints will be analysed on the full analysis set. The proportion of patients achieving clinical FIX response at Week 26, will be summarised for the patients who received the terminal dose level. For the primary analysis, last-observation-carried forward (LOCF) methodology will be used to impute any missing FIX value at Week 26, by the last non-missing FIX value. For the primary analysis, FIX response will be assessed whether or not the patient is receiving or has been receiving immunosuppressants due to transaminitis outside the period of prophylactic immunosuppressant treatment. The proportion of patients also achieving normalization will be summarised on a similar manner.

Sensitivity analyses of the primary endpoint will be performed:

- The proportion of patients achieving clinical or normalised FIX response at Week 26 with patients with a missing FIX response at Week 26 being considered as a non-responder.

- The proportion of patients achieving clinical or normalised FIX response at Week 26 excluding those patients who have received immunosuppressants in the previous 28 days (4 weeks) before the Week 26 assessment, will also be summarised.
- The proportion of patients achieving clinical or normalised FIX response at Week 26 excluding those patients with major protocol deviations (per-protocol set) will also be summarised.

More details and additional sensitivity analyses may be provided in the statistical analysis plan.

14.7 Secondary Endpoints

14.7.1 Safety

Descriptive statistics (number of observations, mean, standard deviation, minimum, median, and maximum values) 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. In particular, patients achieving FIX activity above 150% of normal will be summarised by dose and overall.

Vital signs (systolic and diastolic blood pressure, temperature, and pulse) and physical examination will be summarised by dose level using appropriate descriptive statistics. Continuous variables will be summarised using number of observations, mean, standard deviation, minimum, median, and maximum values. Categorical values will be summarised using number of observations and percentages.

14.7.2 Endogenous FIX Production

The FIX response will be derived for each patient based on FIX activity level measured at the central laboratory. Baseline FIX activity level will be defined as described in section 11.4.2.1.

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, will be summarised by dose and overall.

The proportion of patients achieving FIX activity at or above 5%, 15%, 30%, 40%, 50%, 70% and 150% of normal, at each scheduled visit, will be summarised by dose and overall.

Change from baseline in FIX activity as a percentage of normal values will be summarised in tabular and graphical format for each patient, by dose and overall.

14.7.3 Haemostatic Effectiveness

Annualised Bleeding Rate (ABR)

The number of breakthrough bleeding episodes (spontaneous and traumatic) following FLT180a infusion will be annualised, and compared with the patient's own baseline bleeding history (the annualised mean of 3 years of historical bleeding records, and, where possible, prospective data collected during the screening period will be used).

Factor IX Concentrate Consumption

The dose (IU/kg) of factor concentrate used overall and by type of bleeding episode (i.e., spontaneous or traumatic) and location of bleeding episode (i.e., joint, soft tissue, or muscle) will be summarised with descriptive statistics. The total units of annualised factor consumption will be calculated and compared with the patient's own baseline factor concentrate history (the annualised mean of 3 years' historical factor concentrate consumption records, and, where possible, prospective data collected during the screening period will be used).

In order 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 will be from day 15 inclusive to the date of completion of the last diary. More details will be provided in the SAP.

14.7.4 **Shedding**

Clearance of vgs in plasma, saliva, urine, stool, and semen will be summarised. The time to unquantifiable result by body fluid will be summarized and listed.

14.7.5 **Immune Response**

FIX

Immune response to the FIX transgene product (i.e., development of inhibitors) will be assessed by measurement of the level of inhibitors.

Descriptive statistics (number of observations, mean, standard deviation, minimum, median, and maximum values) will be calculated for immune response laboratory tests at applicable visits.

14.8 Exploratory Endpoints

14.8.1 **Haemostatic Effectiveness**

If data permits, explorations of the correlation between FIX levels and bleeding events will be performed. For example, the proportion of bleeding events (all, treated, spontaneous) by FIX activity range of interest: <5%. 5-30%, 30-50% and >50% will be summarised and graphical displays produced.

14.8.2 **Immune Response**

AAV-S3 Capsid

Immune response to the AAV-S3 capsid will be assessed by measurement of the S3 neutralising antibody titre.

T-cell Responses to AAV-S3 Capsid in Peripheral Blood Mononuclear Cells

Descriptive statistics (number of observations, mean, standard deviation, minimum, median, and maximum values) will be calculated for immune response laboratory tests at applicable visits.

14.8.3 Health Economics

Quality of life will be evaluated using the EQ-5D-5L and Haem-A-QoL.

In addition, disability status will be assessed using the WHODAS 2.0 and the physical activity will be assessed using the HAL 2005. Haemophilia health status will be assessed using PROBE. Joint health and function will be evaluated using the HJHS score.

Descriptive statistics will be applied to study the changes in the scores from baseline to end-of-treatment in each dose level.

Descriptive analyses of the PROBE questionnaire data will be performed separately.

14.9 Subgroup Analysis

Subgroup analysis will be performed where appropriate including:

- Patients receiving FIX on-demand therapy
- Patients on conventional FIX prophylaxis therapy
- Patients on extended half-life FIX prophylaxis therapy
- Baseline FIX level
- Baseline HJHS score.

Additional subgroups may be defined in the SAP.

A DMC will meet to review the safety results from a given cohort before escalating to the next dose in a new cohort. With the agreement or at the recommendation of the DMC, additional patients may be added to any cohort to characterise a DLT or any safety concern that has not met DLT stopping rules or the FIX response.

14.10 Handling of Missing Data

Missing data is expected due to the patient population, and general features of clinical studies:

- (D) patient died
- (W) patient withdrew informed consent or became lost to follow-up
- (M) assessment (may be a complete visit) could not be performed for a reason not covered by one of the reasons above.

Number and percentage (based on the number of treated patients) of missing values and reasons (categories as defined above) will be summarised by visit for FIX levels.

LOCF methodology will be used for the primary efficacy analysis, irrespective of the reason why the data is missing, if known. Sensitivity analysis will be performed as described in section 14.6.2. 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.
- Completely missing: impute first dose date unless the end date suggests it could have started before this, in which case impute the 1st January of the same year as the end date.

Partial/missing end date:

- Missing day: impute the last day of the month unless month is same as month of end of study visit, then impute day prior to end of study visit.
- Missing day and month: impute 31st December unless year is the same as end of study visit, then impute day prior to end of study visit.

14.11 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.

15 Sponsor and Investigator Responsibilities

This study is conducted in accordance with current applicable regulations, ICH, EU Directive 2001/20/EC and its updates; The Medicines for Human Use (Clinical Trials) Regulation SI.2004/1031 and its updates; Advanced Therapy Medicinal Product Regulation 1394/2007/EC or 21 CFR Part 312 in the United States and local ethical and legal requirements.
The name and address of each third-party vendor (e.g., CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

15.1 Sponsor's Responsibilities

15.1.1 Good Clinical Practice Compliance

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 Good Clinical Practice (GCP) Guideline E6 (R2) 2017, EU Directive 2001/20/EC, The Medicines for Human Use (Clinical Trials) Regulation SI.2004/1031 as amended, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organising/managing the research on behalf of the sponsor to inspect study data, patients' medical records, and eCRFs in accordance with current GCP and the respective local and

international government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of investigational product for shipment to the site.

15.1.2 **Monitoring Requirement for the Trial**

The sponsor will determine the appropriate level and nature of monitoring required for the trial. 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 trial.

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

15.1.3 **Insurance**

The following statement applies to all sites excluding USA:

University College London (UCL) holds insurance against claims from participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of UCL or another party. Participants who sustain injury and wish to make a claim for compensation should be advised to do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

The following statement applies to sites in USA:

University College London holds insurance against claims from participants for injury caused by their participation in this clinical trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, if this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical trial. University College London does not accept liability for any breach in the hospital's duty of care,

or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to UCL, upon request.

15.1.4 Public Posting of Study Information

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

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

The CRO on behalf of the sponsor will provide a summary of the clinical study report to the competent authority and, where applicable, the Independent EC of the member state(s) concerned as required by regulatory requirement(s) and to comply with the community guideline on GCP. This requirement will be fulfilled within 6 months of the end of the study completion date for paediatric studies and within 1 year for non-paediatric studies as per guidance.

15.1.6 Study 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 IRBs/ECs 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 CRO will make an end-of-study declaration to the relevant competent authority as required by Article 10 (c) of EU Directive 2001/20/EC and The Medicines for Human Use (Clinical Trials) Regulation SI.2004/1031 as amended.

15.1.7 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 patients of the trial; or

(b) the scientific value of the trial.

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

(a) the conditions and principles of GCP in connection with that trial; or

(b) the protocol relating to that trial, 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 trial conduct phase.

15.2 Investigator's Responsibilities

15.2.1 Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (R2) 2017, EU Directive 2001/20/EC, The Medicines for Human Use (Clinical Trials) Regulation SI.2004/1031 as amended and applicable regulatory requirements and guidelines.

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 patient has a primary care physician, the investigator should, with the patient's consent, inform them of the patient's participation in the study.

15.2.2 Protocol Adherence and Investigator Agreement

The investigator and any co-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those patients 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 their site, the investigator will promptly inform the sponsor and the IRB/EC 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, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicentre studies, the chief Investigator according to national provisions and will be documented in the investigator agreement.

15.2.3 Data Collection Tools and Source Document Identification

Data will be collected from sites on trial-specific eCRFs.

Source data are contained in source documents and must be accurately transcribed on to the eCRF. Examples of source documents are hospital records which include laboratory and other clinical reports etc.

A source document identification list will be implemented prior to the start of the trial to identify:

- which data is to be recorded directly onto the eCRF;

- which data is recorded firstly into source documents, such as medical notes, and then transcribed into the eCRF; and
- which data is not to be recorded in the eCRF but only recorded in source documents, e.g., participant questionnaires and diary cards.

It is the responsibility of the PI to ensure the accuracy of all data entered in the eCRF. The delegation log will identify all those personnel with responsibilities for data collection and handling.

15.2.4 Completing Case Report Forms

All eCRFs must be completed and signed by staff that are listed on the site staff delegation log and authorised by the PI to perform this duty. The PI is responsible for the accuracy of all data reported in the eCRF.

15.2.5 Data Handling and Analysis

A trial-specific data management plan will be in place for the trial. 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, database lock and data transfers.

15.2.6 Monitoring

The investigator must permit authorised representatives of the sponsor, the respective national, local, or foreign regulatory authorities, the IRB/EC, and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The clinical research associate/study monitor (and auditors, IRB/EC or regulatory inspectors) may check the eCRF entries against the source documents.

15.2.7 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US Food and Drug Administration (as well as other US national and local regulatory authorities), the European Medicines Agency the Medicines and Healthcare Products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

15.3 Ethical and Regulatory Considerations

15.3.1 Informed Consent

It is the responsibility of the investigator to ensure that appropriate informed consent has been obtained from all study patients prior to any study-related procedures. Signed ICFs must remain in each patient's study file and must be available for verification at any time.

The PI will provide the sponsor with a copy of the ICF that was reviewed by the IRB/EC and which received their favourable opinion/approval. A copy of the IRB/EC's written favourable opinion/approval of these documents must be provided to the sponsor, prior to 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 chief Investigator) is

responsible for this action. Additionally, if the IRB/EC requires modification of the sample patient information and ICF provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

15.3.2 Institutional Review Board or Ethics Committee

It is the responsibility of the PI(s) to ensure that all aspects of institutional review are conducted in accordance with current governmental regulations.

The sponsor must receive a letter documenting EC approval, which specifically identifies the protocol, protocol number, and ICF, prior to the initiation of the study. Protocol amendments will be subject to the same requirements as the original protocol.

A progress report must be submitted to the EC at required intervals and not less than annually.

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

15.3.3 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 IRB/EC 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 participants, see Section 13.2.13 Reporting Urgent Safety Measures. Other local or national approvals may be required and will be sought accordingly prior to implementation.

15.4 Record Keeping and Archiving

At the end of the trial, all essential documentation must be archived securely by the chief investigator and trial sites according to ICH GCP requirements.

Essential documents are those which enable both the conduct of the trial and the quality of the data produced to be evaluated and show whether the site complied with the principles of GCP and all applicable regulatory requirements.

In accordance with regulations for ATIMPs, certain items must be archived for a 30-year period (see Section 15.4.1). All archived documents must continue to be available for inspection by appropriate authorities upon request

Essential documents must be maintained and may not be destroyed without written permission from the sponsor.

15.4.1 Archiving Essential Trial Documentation Relating to Traceability

In accordance with the Advanced Therapy Regulations (1394/2007/EC), all parties (the sponsor of the trial, the tissue establishments/procurement organisation (if applicable), the animal facility (if applicable), the manufacturer and the investigator/institution where the ATIMP is used) will keep their parts of the traceability records for a minimum of 30 years after the expiry date of the ATIMP, or longer if required by the terms of the clinical trial authorisation or

by the agreement with the sponsor. These requirements will be set out in contractual agreements between the parties and the sponsor.

To comply with the regulatory requirements, each responsible party must ensure that the information relating to the traceability and accountability, from the production of ATIMPs to the participant receiving the ATIMPS are archived for a minimum 30 years after the expiry date of the ATIMP.

The following essential documents/traceability data will be retained by the investigator and institution responsible for the human application of the ATIMP:

Shipping Records for IMP:

- Certificate of analysis of the IMP
- Treatment allocation and decoding documentation
- Participant identification code list
- IMP accountability at the site including final disposition of both used and unused product.

These records contain relevant information for traceability purposes and at least the following minimum data set from these records will be kept for 30 years after the expiry date of the product, or longer if required by the terms of the clinical trial authorisation or by the agreement with the sponsor:

- Identification of the investigator/institution
- Identification of the sponsor
- Identification of the manufacturing site
- Product name/code
- Pharmaceutical form, route of administration, quantity of dosage units and strength
- Batch and/or code number
- Trial reference code
- Trial participant code
- Participant identification code list (links name of recipient to the trial participant code)
- Product expiry/retest date
- Date of administration
- Records of any product that was unused or destroyed at site and its final status

The participant medical records will contain the product name/code, the trial reference code, trial participant code and administration dates and dose in order to ensure that a link can be made back to the identity of the product and the further traceability records of the investigator and sponsor.

15.5 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 patients' identities. UCL as Sponsor is the Data Controller as defined under the EU General Data Protection Regulation 2016 and Data Protection Act 2018.

Patients are assigned a unique identifying number.

The results of studies – containing patients' unique identifying number and relevant medical records will be recorded. They 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.

16 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 multicentre study, the first publication of the study results shall be made by the sponsor in the form of a multicentre publication of the compiled and analysed study results. If such a multicentre 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 multicentre study publication of the study results, an investigator may individually publish the study results from the 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 PI shall withhold submission of such publication for up to an additional 60 days to allow for filing of a patent application.

17 Funding

The trial is fully funded by Freeline Therapeutics Ltd.

18 Statement of Compliance

The trial will be conducted in compliance with the approved protocol, ICH GCP and applicable regulatory requirement(s).

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Appendix 1: Treatment Management Plans

1.1 Management of Thrombosis

Patients will be informed in advance of potential early symptoms and signs of thrombotic phenomena, including pain and/or tenderness along a vein, swelling of an arm or leg without pain or tenderness, redness along a vein, low fever without any known reason (such as a cold or flu), sudden shortness of breath or difficulty breathing or coughing, sudden chest pain, sudden severe headache or changes in vision, and numbness or tingling in arms or legs. If such an event occurs while the patient is at home, the patient will be instructed to seek immediate medical care.

In the event of a thrombotic event, it will be managed according to established guidelines:

Prevention and treatment of venous thromboembolism: international consensus statement (guidelines according to scientific evidence). Clin. Appl. Thromb. Hemost. 2013 Mar-Apr;19(2):116-231.

1.2 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 centre for at least 12 hours following infusion to minimise 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 infusion and then every 2 hours for 6 hours.

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.

1.3 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.

1.4 Management of Inhibitors

Studies in non-human primates have shown that immune modulatory treatment with rituximab and cyclophosphamide is effective at managing neutralising anti-human FIX antibodies following AAV mediated gene transfer.[‡] This finding has been replicated in recent studies resulting in complete eradication of the neutralizing anti-hFVIII antibody in two animals and a partial reduction in antibody titre in another. Therefore, should a neutralising antibody response

to the transgenic protein be observed in patients in this study a rituximab based immunosuppressive regimen will be implemented.

‡Nathwani AC, Gray JT, McIntosh J et al. Safe and efficient transduction of the liver after peripheral vein infusion of self complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates. Blood. 2007;109:1414-1421.

1.5 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 centre for at least 12 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 following infusion and then every 2 hours for 6 hours. Should it occur, infusion reaction 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.

Appendix 2: 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 haematopoietic stem cell transplantation (Atabani et al., 2012; Lim et al., 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 et al., 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 et al., 2016; Natori et al., 2018).

A CMV PCR titre > 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 UCL15/0552, 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 et al., 2017). In which case we suggest one of the following: Valganciclovir 900 mg bd orally, Ganciclovir 5mg/Kg bd iv or Foscarnet 60mg/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

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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

Appendix 3: Management Guidelines for Tacrolimus Dosing

Introduction

Tacrolimus, is a macrolide immunosuppressant produced by Streptomyces tsukubaensis 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). 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.

Target tacrolimus whole blood trough level

Target trough levels (equivalent AUC of 200 ng.h/mL) are approximately 15 ng/ml.

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.

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. Owing to 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 stabilising 48 to 72 hours after the dose adjustment is made. In clinical practice, 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 1: 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:

Table 2: Suggested dose adjustments based on trough levels Day 2/3.

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

Appendix 4: Summary of Protocol Changes

Version 1.0 to 2.0

Section	Page Number(s)	Summary of Change
5.2	21	Detailed text for non-clinical summary has been deleted. Full reference for non-clinical data now resides in the investigator brochure.
5.3	22	Administrative changes to phrasing of text for clarity.
5.4.1	24-25	Amended text related to dose justification.
5.4.2	29	Under Heading Thrombogenicity. Inclusion of data relating to findings in GLP toxicology program.
	26-30	Administrative changes to phrasing of text for clarity.
	30	Under Heading Risks associated with DNA impurities. Removal of redundant text related to non-clinical studies.

Version 2.0 to Version 3.0

Section(s)	Page Number(s)	Summary of Change
Title Page	1	Update to version number and addresses of CI and Sponsor.
2	9, 10	List of Abbreviations updated where required in line with content of protocol.
3	11	Trial Personnel contact details updated where required.
4, 14.3	12, 69	Increase of maximum number of patients that maybe enrolled from ‘18’ to ‘24’.
4, 12.3.3, 14.3	12, 14, 62, 63, 69	Increase in number of patients to be enrolled at the terminal dose level from ‘6’ to ‘14’.
4, 12.3.2, 12.3.3, 14.9	12, 13, 62, 63, 72	Removal of the specified maximum of 6 patients can be treated at a single dose level. Additional patients (number unspecified) can be added at any dose level at the request of the trial management group and data monitoring committee and at discretion of the Sponsor.
4, 5.1	13, 23	Addition of sentence to Rationale ‘Even with extended half-life (EHL) factor concentrates frequent infusions are required for the life time of the patient.’
4, 5.4.1	13, 26	Update in potential disease phenotype from ‘milder forms’ to ‘mild/normal’.
4, Table 3, 12.3.1	13, 27, 61	Update in dose of Cohort 3 (high dose) from ‘6 x10^{12} vg/kg’ to ‘4 x 10^{12} vg/kg’.
4, 5.4.1, 7.1, 12.3.1, 12.3.3	13, 27, 35, 61, 62, 63	Update to reflect that the dose level of cohort maybe reduced dependent on the FIX response seen, to avoid exposing patients to levels exceeding the normal physiological range. Dose reduction steps are specified.
4, 5.4.1, 7.1, 12.1.2, 12.3.2, 12.3.3	13, 14, 27, 35, 36, 60, 62, 63	Update to provide clarity and correct omissions that safety and efficacy outcomes and dose escalation/reduction and selection of terminal dose level will be over seen by ‘a trial management group and a data monitoring committee’ not just ‘a data monitoring committee’.
4, 5.4.1, 12.3.3	13, 27, 62, 63	Update to the goal for FIX activity from ‘40-100%’ to ‘70-150%’. FIX activity of 70-150% representing normalisation and a level at which patients may undergo surgical procedures without a requirement for exogenous factor concentrates.
4, Table 1, 5.4.1, 5.4.2, 7.1, 9.3, 9.4, 11.2.1	13, 18, 19, 20, 28, 29, 30, 36, 42, 43, 44, 49	Update to the corticosteroid regimen from ‘6’ to ‘8’ weeks and a change from ‘weeks 6-12’ to weeks ‘weeks 4-12’.
4, 8.1	14, 38	Update in Inclusion Criteria 2, a, ii from ‘On-demand therapy with current a or past history of 4 or more bleeding episodes/year, or’ to ‘On-demand therapy with a history of 4 or more bleeding episodes/year on average over the past 3 years, or’.
4, 8.1	14, 38	Update in Inclusion Criteria 5 from ‘Lack of neutralising anti-AAV-S3 antibodies using an *in vivo* transduction inhibition assay’ to ‘Lack of neutralising anti-AAV-S3 antibodies using an *in vivo* transduction inhibition assay within 4 weeks of vector administration’.
4, 5.4.2, 8.2	14, 32, 39	Addition of an Exclusion Criteria 2 (previous Exclusion Criteria #’s from 2 onwards, increased by 1) ‘Patients at high risk of thromboembolic events (high risk patients would include those with a history of arterial or venous thromboembolism (e.g. deep vein thrombosis, pulmonary embolism, non-haemorrhagic stroke, arterial

		embolus) and those with known inherited or acquired thrombophilia including conditions such as atrial fibrilation)'.
4, 8.2	14, 39	Update in Exclusion Criteria 6 (previously 5) from 'Evidence of liver dysfunction (persistently elevated alanine transaminase >1.5 x upper limit of normal)' to 'Evidence of liver dysfunction (persistently elevated alanine aminotransaminase, aspartate aminotransferase, bilirubin >1.5 x upper limit of normal)'.
4, 8.2	14, 39	Update in Exclusion Criteria 10 (previously 9) from 'Patients with uncontrolled cardiac failure or unstable angina' to 'Patients with uncontrolled cardiac failure, unstable angina or myocardial infarction in the past 6 months'.
4, 5.4.2, 8.2	15, 30, 39	Update in Exclusion Criteria 13 (previously 12) from 'Known or suspected intolerance or hypersensitivity to the investigational product of its excipients' to 'Known or suspected intolerance, hypersensitivity or contraindication to the investigational product and non-investigational medicinal products or their excipients'.
4, 8.2	15, 39	Addition of an Exclusion Criteria 14 'Planned major elective surgery prior to the end of trial'.
4, 7.2.2, 14.7.1	15, 16, 37, 71	Addition of Secondary endpoint, Endogenous (hFIX) production of 'The proportion of patients achieving hFIX activity at or above 70% of normal at week 26' and updates to applicable sections of statistical analysis section.
4, Table 1, 7.2.3, 11.1.1, 11.4.4.1, 14.8.1	15, 18, 19, 20, 37, 46, 56, 72	Addition of the 'EQ-5D-5L' patient report outcome to the study. Reflected in Scheduled of Assessments, trial design, study procedures and statistical analysis sections.
Table 1, 11.1.1, 11.1.2, 11.2.1, 11.2.1.1, 11.3, 11.4.2.3, 11.4.3.2, 11.4.3.5, 11.4.3.6	18, 19, 20, 21, 22, 45, 46, 47, 48, 49, 50, 51, 53, 54, 55	Updates to 'Table 1: Schedule of Assessments' to split out the requirements for the Infusion Week (Day -1 to Day +4) by creation of a second table 'Table 2: Detailed Schedule of Assessments for Infusion Week'. Wider formatting/order/ rationalisation of assessments listed and adjustment of table footnotes for accordingly. Implicated changes in section 11, Study Procedures.
Table 1, Table 2, 7.1, 11.1.2	18, 19, 20, 21, 22, 34, 46	Addition of a window to the Day -1 visit which may now be conducted as early as Day -3 for logistical reasons.
Table 1, Table 2	18, 19, 20, 21, 22	Addition of a window to the Day +4 visit which may now be conducted +/- 1 day.
Table 1, 11.1.1, 11.4.1.5	18, 19, 20, 45, 52	Addition of Target Joint Assessment at Screening visit.
Table 1, 11.2.1, 11.3 11.4.3.2	18, 19, 20, 49, 50, 55	Addition of Liver Function Test (Local) at Week 14, Week 16, Week 20 and Week 26/EOS visits.
Table 1, 11.2.1, 11.3, 11.4.2.3	18, 19, 20, 49, 50, 53	Addition of hFIX Activity Level (Local) at Week 14, Week 16, Week 20 and Week 26/EOS visits.
Table 1, Table 2, 11.4.1.6, 11.4.3.4	18, 19, 20, 21, 22, 52, 55	Addition of requirement for primary and back-up samples for AAV Antibody Screen, hFIX Activity Level (Central) and AAV-S3 Antibody Titre.
Table 1, 11.1.1, 11.4.1.6	18, 19, 20, 45, 52	Confirmation that AAV Antibody Screen is via transduction inhibition assay and that repeat testing may be indicated during long screening period.
Table 1, Table 2	18, 19, 20, 21, 22	Liver Function Test (Local) to include 'Albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase, aspartate aminotransferase' added to Schedule of Assessments for clarity.
Table 1, 11.2.1.1	18, 19, 20, 49, 50	During intensive monitoring period for Liver Function Tests (Local) and hFIX Activity Level (Local) a recommendation on how the test should be distributed through the week. 'Three times per week. The tests should be as evenly spaced through the week as possible, for example: Monday, Wednesday and Friday.'

		'Twice weekly. The tests should be as evenly spaced through the week as possible, for example: Monday and Thursday or Tuesday and Friday.'
Table 2, 11.1.2	21, 22, 46	Clarification that the pre-infusion consent may occur prior to Day -1.
Table 2	21, 22	Haematology, Chemistry incl. CRP, Coagulation Screen (Local) to include 'Complete blood count with differentials, sodium, potassium, phosphate, blood urea nitrogen, serum creatinine, CRP, prothrombin time, activated partial thromboplastin time' added to Schedule of Assessments for clarity.
5.3, 5.4.1, 5.4.2, 7.1, 12.3.3	25, 28, 35, 36, 62, 63	Addition of text describing outcomes from the George et al, NEJM 2017 paper around the asymptomatic increase in ALT been seen as early as week and the FIX activity levels at week 4 representing ~75% of levels seen at steady state.
5.4.1	27	Addition of '*In vivo* data in mice models are not predictive of transgene expression in humans and therefore the proposed doses have been estimated based on extrapolation of *in vitro* data in human cells comparing the performance of elements of the AAV2/8-LP1-hFIXco construct used in the previous study by Nathwani et al. and those incorporated in FLT180a.[11,15,16,17] Please refer to the investigator brochure for full details.' to support rational for dose selection.
5.4.1	28	Supplementary updates to the data reported in the protocol from the pre-clinical work.
5.4.1, 7.1., 12.3.3	27, 28, 35, 36, 62, 63	Updates to the dose escalation rules, selection of target dose criteria and criteria for dose reduction.
5.4.1	28	Addition of 'Analysis of the data from the studies conducted by Nathwani et al[11,17] has shown a marked effect of corticosteroid treatment on FIX activity levels. An increase of 2.5 to 3-fold in FIX levels have been observed in parallel with the prednisolone regimen (unpublished data), these return to pre-steroid levels approximately 5 weeks following cessation. The steroid response with the smaller [redacted] promoter used in FLT180a is expected to be less profound but not absent and therefore pre-steroid FIX levels (week 4) will be used to guide dose escalation decisions.' to support rationale for dose escalation.
5.4.2	32	Addition of 'The World Federation of Hemophilia (WFH) regard 150% as the upper limit of normal for Factor IX activity.' to support risk/benefit review for thrombogenicity.
5.4.2	33	Supplementary updates to the data reported to support risk/benefit review for Risk associated with DNA impurities.
7.1, 10.3	35, 45	Update in guidance on when prophylactic factor concentrate maybe ceased in line with clinical practice, from 'Only after FIX activity levels ≥3% are reached at 2 consecutive measurements will the patients be asked to stop their prophylactic FIX treatment.' to 'If FIX activity levels ≥3% are reached then prophylaxis will be held pending a repeat analysis within a period of 72 hours. If the FIX activity levels are ≥3% at that time then prophylaxis will be stopped with continued/regular assessment of FIX activity levels and occurrence of spontaneous bleeding.'
Figure 1	36	Removal of Figure 1: Study Schematic.
9.2.4	41	Removal of 'on a per patient basis' so number of amount of IMP that can be shipped to infusion site is not limited to allow some flexibility for scheduling.
9.3	42	Update in tablet doses available of prednisolone.
10.2	44	Addition of 'time allowing, i.e. investigator permission not required for emergency medical care.' as previous wording could have implied this was not allowed.
11.4.1.2	51	Update to reflect correct method of derivation of the patient number.
11.5	57, 58, 59	Volume of blood to be drawn from each patient updated to reflect updated amounts that will be required.
12	59	Addition of 'The sponsor will retain final responsibility for decision making on all aspects of dose progression, dose escalation/reduction and stopping rules.'
12.3.2	62	Update from 'If DLT observed in third patient, apply temporary halt to trial and consult competent authority.' to 'If DLT observed in third patient and this is second patient in which a DLT has been observed, apply temporary halt to trial and consult competent authority.' for clarification.
12.5	64	Removal of stopping rule 'Persistence of vgs in semen >3 months'.
13.2.11	68	Update from 'The CRO is responsible for notifying the relevant regulatory authorities and Central Ethics Committees(ECs)/Institutional Review Boards (IRBs) of related, unexpected SAEs.' to 'The CRO is responsible for notifying the relevant regulatory authorities and Central Ethics Committees(ECs)/Institutional Review Boards (IRBs) 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 CRO learning of them with any additional relevant information reported within a further 8 days. Other SUSARs must be reported

		within 15 days from the CRO learning of them. The CRO will also inform all participating sites.'
14.10	72	Update from 'Partial/missing end date: • Missing day: impute the last day of the month unless month is same as month of last dose of study drug, then last dose date. • Missing day and month: impute 31st December unless year is the same as end of study visit, then impute day prior to end of study visit.' to 'Partial/missing end date: • Missing day: impute the last day of the month unless month is same as month of end of study visit, then impute day prior to end of study visit. • Missing day and month: impute 31st December unless year is the same as end of study visit, then impute day prior to end of study visit.' to correct inaccuracies.
15	73	Addition of 'Advanced Therapy Medicinal Product Regulation (1394/2007/EC) or 21 CFR Part 312 in the United States'.
15.3.3	77, 78	Addition of section 15.3.3 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 IRB/EC 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 participants, see Section 13.2.13 Urgent Safety Measures. Other local or national approvals may be required and will be sought accordingly prior to implementation.
15.5	79	Addition of 'UCL as Sponsor is the Data Controller as defined under the EU General Data Protection Regulations 2016' and removal of any references that initials or date of birth will or might be collected.
19	81, 82, 83	Duplication of references noted and updated, numbering addressed. Due to Endnote autoformatting issues, difficult to retain integrity of structure of document with full tracked changes of updates to numbering. All new references and deleted references indicated as such in tracked changes version.
All	All	Appropriate spelling, grammatical, formatting and minor updates and addition/removal/update of single words for clarity/consistency of text throughout protocol.

Version 3.0 to Version 3.1

Section(s)	Page Number(s)	Summary of Change
Title Page	1	Update to version number.
4, 8.2	14, 39	Update in Exclusion Criteria 2 from 'Patients at high risk of thromboembolic events (high risk patients would include those with a history of arterial or venous thromboembolism (e.g. deep vein thrombosis, pulmonary embolism, non-haemorrhagic stroke, arterial embolus) and those with known inherited or acquired thrombophilia including conditions such as atrial fibrilation)' to 'Patients at high risk of thromboembolic events (high risk patients would include those with a history of arterial or venous thromboembolism (e.g. deep vein thrombosis, pulmonary embolism, non-haemorrhagic stroke, arterial embolus) and those with acquired thrombophilia including conditions such as atrial fibrilation)'.

Version 3.1 to Version 4.0

Section(s)	Page Number(s)	Summary of Change
Title Page	1	Update to version number/date and addition of IND#.
4, Table 1, 5.4.1, 5.4.2, 7.1, 9.3, 9.4, 11.2.1, 11.2.1.1	13, 17, 18, 19, 28, 29, 32, 35, 41, 42, 43, 44, 49, 50	Extensive updates to required corticosteroid prophylaxis regimen and actions in response to elevations in ALT rises. Associated updated to LFT monitoring and test of reactivation of hepatitis.

Table 1, 11.2.1, 11.3, 11.4.6.5, 11.5	17, 18, 19, 49, 50, 57, 58	Addition of samples for 'FIX activity research sample' and applicable updates to study schedule and study procedures/assessments sections.
Table 2, 11.4.3.2	20, 21, 54	Correction of error to reinstate 'platelets' as a specified test within the haematology local laboratory panel.
5.4.1	29	Update of sentence 'In macaques, neutralising antibodies have been observed in a small proportion of animals exposed to human GTMP' to 'In macaques, neutralising antibodies have been observed in a small proportion of animals exposed to human GTMP, including FLT180a.'
9.2.2	39	Update to correct referenced regulation from 'Annex 13 (EudraLex Volume 4) in the EU' to '(EudraLex Volume 4 GMP, Part III Annex 13 and Part IV in the EU)'.
11.1.1	46	Addition of 'A patients alcohol intake for the course of the trial should also be discussed at the screening visit with a recommendation to moderate their intake for the duration of the trial. This discussion should be ongoing throughout the trial.'.
11.1.2	47	Correction in error which specifies a 200 mL infusion volume of the vector. Adjusted to 'Calculated FLT180a vector dose…'.
11.4.4	56	Addition of section on 'Vector Shedding' to study procedures and assessments section which has been previously omitted in error.
11.5	58	Adjustment of 'Volume of blood to be drawn from each patient' to reflect addition of FIX activity research plasma sample and back-up sample collection for routine FIX activity sampling which was omitted in error.
12.3.3, 12.5, 13.2.9	63, 64, 68	Updates to clarify definition with regards to confirming inhibitor and associated impact should an inhibitor be identified.
13.2.12	69	Addition of the following 'Expedited reporting and reporting of adverse events will be in accordance with EudraLex Volume 10, Chapter II (Detailed Guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3'; 2011/C 172/01), June 2011) in the EU and with 21 CFR Part 312 to the FDA.'.
19	81, 82, 83	Addition of supporting references.
All	All	Appropriate spelling, grammatical, formatting and minor updates and addition/removal/update of single words for clarity/consistency of text throughout protocol.

Version 4.0 to Version 5.0

Section(s)	Page Number(s)	Summary of Change
Title Page	1	Update to version number.
4, Table 1, 5.4.1, 7.1, 9.4, 11.2.1, 12.3.3	13, 17, 18, 19, 20, 28, 29, 30, 36, 43, 44, 50, 63, 64	Update to required corticosteroid prophylaxis regimen to commence at Week 3 and test for reactivation of hepatitis. Update to actions to be taken in response to break-through transaminitis.
Table 1, 11.2.1.1, 11.4.3.2, 11.5	17, 18, 19, 20, 50, 55, 59, 60	Addition of local haematology samples to be conducted in line with regular local LFT monitoring.
Table 1, 11.2.1, 11.3, 11.4.6.4, 11.5	17, 18, 19, 20, 50, 51, 58, 59, 60	Update to reflect collection of Research Plasma Samples at study visits with no dependency on an elevation in ALT.
12.2	61	Update to definition for DLT from 'A DLT is defined as any grade 3 or greater AE at least possibly related to FLT180a.' to 'A DLT is defined as any grade 3 or greater AE at least possibly related to FLT180a except for increases in ALT or AST that are not associated with increases in bilirubin.
5.4.1, 12.3.3	28, 63, 64	Updates to reflect that Week 3 values (last point pre-steroid from V5 of protocol) represents ~65% of steady state values and proportional updates to the guide values provided in the dosing rules.

All	All	Appropriate spelling, grammatical, formatting and minor updates and addition/removal/update of single words for clarity/consistency of text throughout protocol.

Version 5.0 to Version 6.0

Section(s)	Page Number(s)	Summary of Change
Title Page	1	Update to version number.
4, 6.1, 7.1, 7.2.1, 14.6.2	12, 14, 15, 16, 37, 39, 40, 76, 77	Addition of a primary efficacy objective 'To assess Factor IX FIX levels following systemic administration of FLT180a, at the terminal dose level' and applicable associated updates to overall design, endpoints and statistical sections.
4, 6.2, 6.3, 7.2.2, 7.2.3, 14.7.5, 14.8.1	12, 15, 17, 37, 40, 78	Removal from secondary objective and addition to exploratory objective 'To assess the immune response to the adeno-associated virus (AAV)-S3 capsid proteins following systemic administration of FLT180a' and applicable associated updates to endpoints and statistical sections. Correction to provide clarification in ambiguity in previous versions of protocol of where this objective should sit.
4, Table 1, Table 2, 6.3, 7.2.3, 11.1.1, 11.1.2, 11.3, 11.4.5.4, 14.8.2	12, 15, 18, 19, 20, 21, 22, 23, 24, 37, 41, 50, 51, 55, 61, 78	Addition of the PROBE patient reported outcome to the study and applicable associated updates to objectives, endpoints, study schedule, study procedures/ assessments and statistical sections.
4, Table 1, Table 2, 7.1, 11.1.1, 11.1.2	13, 14, 18, 19, 20, 21, 22, 23, 24, 38, 48, 49, 50, 51, 52	Extension of the screening period from 'up to 14 weeks' to 'up to 52 weeks' and applicable associated updates to study schedule and study procedures/ assessments sections
4, 7.2.2, 14.6.1, 14.7.1	15, 16, 40, 76, 77	Update of general safety endpoints from primary to secondary and applicable associated updates to statistical section. Correction to provide clarification in error in previous versions of protocol.
4, 7.2.2, 14.7.2	15, 16, 40, 77	Update to endogenous FIX production secondary endpoints and applicable associated updates to statistical section. Updates further to addition of primary efficacy endpoint.
4, 8.2	14, 43	Addition of exclusion criteria 15 'Current or relavant history of a physical of psychiatric illness or any medical condition that in the opinion of the investigator could affect the patients safety or interfere with the study assessments'.
4, 13.2.1, 14.6.1	14, 16, 71, 76	Update to CTCAE version from V4.03 to V5.0.
4, Table 1, Table 2, 7.2.3, 11.1.2, 11.3, 11.4.5.5	15, 18, 19, 20, 21, 22, 23, 24, 41, 51, 55, 61	Update to Health resource utilisation endpoints from 'Number of haemophilia related medical appointments' to 'Number of haemophilia related medical appointments and medical activities' and Number of physiotherapy sessions' to 'Number of physiotherapy sessions, specialist consultations and appointments with professional caregivers' and applicable associated updates to the study procedures/assessments section. Clarifications also made to confirm when health resource utilisation data should be collected and the periods under review at each time point with updates to the study schedule, study procedures/assessments sections.
Table 1, 5.4.2	18, 19, 20, 21, 31, 32	Updates to requirements around confirmation of liver health prior to dosing with FLT180a and applicable associated updates to the study schedule and risks and benefits sections.
Table 1, 11.1.1, 11.4.1.7, 11.5	18, 19, 20, 21, 49, 57, 63, 64	Update to include HCV RNA viral load testing at screening as required with updates to the study schedule and study procedures/assessments sections.
Table 1, Table 2, 11.1.2, 11.2.1, 11.3, 11.4.6.3, 11.4.6.4	18, 19, 20, 21, 22, 23, 24, 51, 54, 55, 62	Updates to provide clarity that the Mononuclear Cells (Research) and Research Plasma Samples are 'Optional' samples in the trial with updates to the study schedule and study procedures/assessments sections.

Table 1, Table 2, 11.1.2, 11.2.1, 11.3, 11.4.6.6, 11.5	18, 19, 20, 21, 22, 23, 24, 51, 54, 63, 64	Addition of Immune Response Research Plasma Samples at Day -1 and EOS and to be taken throughout the trial in response to breakthrough transaminitis with updates to the study schedule and study procedures/assessments sections.
Table 1, Table 2, 5.4.2, 11.2.1, 11.3, 11.4.4	18, 19, 20, 21, 22, 23, 24, 33, 54, 55, 61	Update in requirement of negative vector shedding samples required per sample type from '2' to '3' and associated updates in study schedule, risk/benefit and study procedures/assessments sections.
Table 1, 5.4.2, 9.3, 9.4, 11.2.1, 11.4.3.2	18, 19, 20, 21, 32, 33, 46, 47, 54, 60	Updates around prophylactic regimen and actions in response to breakthrough transaminitis to include: Updates from 'corticosteroid(s)' to 'immunosuppressant(s)' where required; confirmation of time points for HCV reactivation testing; addition of risk language and precautions in relation to the use of tacrolimus; adjustments to when tacrolimus should be utilised in response to breakthrough transaminitis (both initiation and cessation); CMV PCR testing and tacrolimus level testing during tacrolimus treatment. Updates with study schedule, risk and benefits, IMP/NIMP and study procedures/assessments sections.
5.4.1	30	Updated language in relation to preclinical data and related to updates included in Investigator Brochure V3 Addendum 1.
5.4.2	34	Update in language around 'Insertional mutagenesis and/or tumorigenesis' to provide background to patient information sheet language requested by St Jude's, USA IRB.
11.1.1	51	Update to language around alcohol consumption to advise on period of abstinence alongside the moderation already noted.
13.2.9	73	Addition of thrombosis as an Important Medical Event.
14.5.2	76	Clarification of wording to define per protocol population.
14.9	78, 79	Addition of subgroups for analysis.
14.10	79	Addition of update process for handling of missing data.
19	88, 89, 90	Addition of supporting references.
All	All	Appropriate spelling, grammatical, formatting and minor updates and addition/removal/update of words for clarity/consistency of text throughout protocol.

Version 6.0 to Version 7.0

Section(s)	**Page Number(s)**	**Summary of Change**
Title Page	1	Update to version number.
4, Table 1, 5.4.1, 7.1, 9.4, 11.2.1, 14.6.2	13, 16, 18, 19, 20, 21, 31, 32, 36, 39, 46, 47, 48, 54, 78	Updates around prophylactic regimen and actions in response to breakthrough transaminitis to include: Updates from 'corticosteroid(s)' to 'immunosuppressant(s)' where required resulting from inclusion of tacrolimus in the prophylaxis regimen; confirmation of time points for HCV reactivation testing; adjustments to CMV testing and tacrolimus level testing during tacrolimus treatment. Updates with study schedule, risk and benefits, IMP/NIMP and study procedures/assessments sections.
4, 8.2	14, 42	Update in Exclusion Criteria 5 from 'Serological evidence of HIV-1 who have CD4 counts ≤200/mm^3. Patients who are HIV-positive and stable, with an adequate CD4 count (>200/mm^3) and undetectable viral load measured twice in the 6 months prior to enrolment, on an antiretroviral drug regimen are eligible to enrol' to 'Serological evidence of HIV-1'.
4, 8.2	14, 43	Addition of exclusion criteria 16 'CMV IgG postive patients who are CMV PCR positive at screening'.
Table 1, 11.1.1, 11.4.1.7, 11.5	18, 19, 20, 21, 49, 50, 57, 58, 64, 65	Addition of CMV testing at screening in order to asses exclusion criteria 16.
Table 2	22, 23, 24	Adjustment of timepoint of '+1 hour after end of infusion' to schedule of assessments which was omitted in error. Procedures section already reflects timepoint.

Table 2, 11.4.3.2	22, 23, 24, 60	Update to required chemistry test from 'blood urea nitrogen' to 'blood urea nitrogen or urea' to allow flexibility and facilitate local site practices.
7.1, 13.2.10	38, 75	Update to correct omissions when updates were made from V5.0 to V6.0 to change from '2' to '3' in requirement of negative vector shedding samples.
7.1, 12.3.3	39, 70	Adjustment to interval window once the terminal dose is selected from '2 weeks' to '48-hours'.
12.2	67	Addition of text to confirm that any DLT should be reported as an SAE.
12.3	67	Addition of dose of $1.5x10^{12}$ vg/kg a dose reduction level in the intermediate dose cohort.
12.3.3	69, 70	Adjustment to dose escalation criteria review criteria, clarification on wording around stabilisation, peak and acceptable upper limit values of FIX activity level.
12.3.3	70	Addition of guidance around consideration of potential thromboprophylaxis when a threshold of FIX activity level is reached,
13.2.9	74	Addition of the following text 'Other events maybe reported at the discretion of the Investigator.' under the definitions for Important Medical Events.
All	All	Appropriate spelling, grammatical, formatting and minor updates and addition/removal/update of words for clarity/consistency of text throughout protocol.

Version 7.0 to Version 7.1

Section(s)	Page Number(s)	Summary of Change
Title Page	1	Update to version number.
Table 1, 9.4, 11.2.1, 11.4.3.2	18, 19, 20, 32, 48, 54, 60	Update to include the following text 'Local LFT and FIX activity level monitoring should occur at least weekly while patient is on immunosuppression (prophylaxis or treatment of breakthrough) and for two weeks following cessation.'.
12.3.1	67	Update to include a dose of 1.3x1012 vg/kg following a request by the Data Monitoring Committee.

Version 7.1 to Version 8.0

Section(s)	Page Number(s)	Summary of Change
Title Page	1	Update to version number.
4, 8.1	16, 45	Correction of number stated in inclusion criteria #4 from 'two' to 'three'. Item not updated in error in previous versions of protocol.
4, 7.2.2, 14.7.3	17, 19, 43, 88	Addition of wording to clarify start point of analysis for secondary endpoints for haemostatic effectiveness. Updates across endpoints and statistics sections.
4, 7.2.3, 14.8.1	17, 44, 88	Addition of an exploratory endpoint 'Haemostatic effectiveness - Exploration of the correlation between FIX levels and bleeding events over time.' Updates across endpoints and statistics sections.
4, 14.7.4	19, 88	Addition of wording around statistical analysis for secondary endpoint around vector shedding. Correction of an omission from previous versions of protocol.
Table 1, Table 2, 11.2, 11.3, 11.4.3.2	20, 21, 22, 23, 24, 25, 26, 27, 57, 58, 59, 60, 61, 62, 63, 64, 68, 69	Adjustment to the frequency and requirements for local haematology samples tested locally. Addition of chemistry incl. CRP and coagulation screen samples to be tested locally at the same time points. Further includes addition of estimated GFR to the local chemistry panel. Updates in study schedule and study procedures/assessments sections.
Table 1, 5.4.2, 9.4, 11.2, 11.4.3.2, Appendix 3	20, 21, 22, 23, 24, 36, 49, 50, 51, 57, 58, 59, 60, 61, 62, 63, 68, 69, 106, 107, 108	Adjustment to the prophylaxis regimen taking tacrolimus out to Week 20. Associated updates include a set of 'Management guidelines for tacrolimus dosing' included as Appendix 3 to the protocol. Other applicable updates include adjustments to patient monitoring to be line with tacrolimus dosing. Updates in study schedule, risk benefits and study procedures/assessments sections.
Table 1, 11.2, 11.4.2.3, 11.4.3.2	20, 21, 22, 23, 24, 57, 58, 59, 60,	Addition of monitoring visits at Weeks 13, 15, 17, 18, 19, 21, 22, 23, 24, 25. Monitoring to occur twice weekly. Monitoring is focused on patient FIX levels and LFT's. Also includes once weekly haematology, chemistry incl. CRP and coagulation screen. Hepatitis testing, tacrolimus level and CMV testing as needed

	61, 62, 63, 67, 68, 69	up to week 20 only. Updates in study schedule and study procedures/assessments sections.
Table 1, Table 2, 11.1.1, 11.1.2, 11.4.3.1	20, 21, 22, 23, 24, 25, 26, 27, 52, 54, 68	Addition of neck, waist and hip circumference measurements along with bioimpedance to understand patient body morphology. Updates in study schedule and study procedures/assessments sections.
Table 1, 5.4.2	20, 21, 22, 23, 24, 35	Addition of 'elastography' as an optional test should further assessment of the liver be required.
Table 1, 11.2, 11.4.2.3, 11.4.3.2	20, 21, 22, 23, 24, 57, 58, 59, 60, 61, 62, 63, 67, 68, 69	Addition of wording to allow flexibility in location of blood sampling for frequent laboratory assessments (FIX levels and LFT's) and assessments at additional visits (Weeks 13, 15, 17, 18, 19, 21, 22, 23, 24, 25 - FIX levels, LFTs, hepatitis reactivation, tacrolimus level and CMV testing) to be covered by home nursing. Updates in study schedule and study procedures/assessments sections.
Table 1, 5.4.2, 9.4, 11.2, 11.4.3.2, Appendix 2	20, 21, 22, 23, 24, 36, 49, 50, 51, 57, 58, 59, 60, 61, 62, 63, 68, 69, 104, 105	Clarification of requirement for CMV testing to reflect only patients with a positive test (IgG) at screening need ongoing testing through trial and not all patients. Also included in the instance of CMV reactivation are a set of 'Management guidelines for CMV reactivation' included as Appendix 2. Updates in study schedule, risk benefits and study procedures/assessments sections.
Table 2, 11.1.2	25, 26, 27, 56	Adjustment of post-dosing monitoring of vital signs to note that the 10 and 12 hour time-points are only required for the first 2 patients at any given dose level and adjustment of the catch all statement to say that patients remain 'hospitalised for 8 hours'. Updates in study schedule, risk benefits and study procedures/assessments sections.
5.4.2	35	Correction of an error in risk text around risk factors of use of corticosteroids.
5.4.2	36	Update to provide clarification of sampling scheduled for vg sampling in line with rest of protocol.
5.4.2, 12.3.3	38, 78	Addition of wording around on-going risk management and oversight of supratherapeutic levels of FIX and thromboprophylaxis.
9.4	51	Removal of wording around steroid sparing regimens to replace the immunosuppressant prophylactic regimen where contraindicated.
9.4	51	Removal of wording around the need for regular monitoring while on immunosuppressant and for two weeks after. This is now covered in the updates to study schedule.
11.2	57, 58, 59, 60, 61, 62, 63	Update to complete section to provide now details as sub-sections for visits as they occur in schedule with required assessments. Visits grouped where appropriate.
11.4.1.1	65	Addition of text to indicate the need for a meeting with a patient advocate is not required for patients once the terminal dose levels has been selected.
11.4.3.2	68, 69	Addition of calcium, APTT and GGT to the chemistry, coagulation screen and LFT panels for central lab testing.
12.3.1	76	Addition of text introducing a cap to the dose that can be administered in the trial. Text added: 'During the dose escalation phase of the trial, review of FIX data at a dose of 1.3 $x10^{12}$ vg/kg, is suggestive of the impact of body weight on expression levels. A dose capping at 90 kg is being introduced at this dose (1.3 $x10^{12}$ vg/kg) to ensure that patients FIX activity levels, at steady state are ideally in the target range.'
All	All	Appropriate spelling, grammatical, formatting and minor updates and addition/removal/update of words for clarity/consistency of text throughout protocol.

Version 8.0 to Version 8.1

Section(s)	Page Number(s)	Summary of Change
Title Page	1	Update to version number.
Table 2, 11.1.2	25, 26, 27, 56	Reversion of changes in V8.0 to adjustment of post-dosing monitoring of vital signs to note that the 10 and 12 hour time-points are only required for the first 2 patients at any given dose level and adjustment of the catch all statement to say that patients remain 'hospitalised for 8 hours'. ALL patients will have vital signs taken to 12 hours post end of infusion and will remain hospitalised for 12 hours.

		Updates in study schedule, risk benefits and study procedures/assessments sections.