Clinical Trial Protocol

Investigational Medicinal Product (IMP):	AAV5-hFIX (adeno-associated viral vector containing a codon-optimized human factor IX gene)
Indication:	Haemophilia B
Phase:	I/II
Title:	A phase I/II, open-label, uncontrolled, single-dose, dose-ascending, multi-centre trial investigating an adeno-associated viral vector containing a codon-optimized human factor IX gene (AAV5-hFIX) administered to adult patients with severe or moderately severe haemophilia B
Short Title:	Phase I/II trial of AAV5-hFIX in severe or moderately severe haemophilia B
Trial ID:	CT-AMT-060-01
EudraCT No.:	2013-005579-42
Date:	20 April 2021
Version:	7.0
Original Protocol	20 June 2014
Protocol Amendment 1	17 July 2014
Protocol Amendment 2	24 November 2014
Protocol Amendment 3	12 December 2014
Protocol Amendment 4	12 January 2015
Protocol Amendment 5	27 March 2015
Protocol Amendment 6	23 September 2015
Protocol Amendment 7	22 December 2015
Protocol Amendment 8	20 April 2021
Sponsor:	uniQure biopharma B.V. Paasheuvelweg 25A 1105 BP Amsterdam The Netherlands
GCP Statement:	This protocol has been designed and will be conducted, recorded, and reported in compliance with the principles of Good Clinical Practice (GCP) guidelines (as are stated in U.S. federal regulations as well as in "Guidance for Good Clinical Practice", International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use), as well as with the detailed guidelines on good clinical practice specific to advanced therapy medicinal products (as issued by the European Commission on 03 December 2009).

Confidentiality statement
This uniQure biopharma B.V. document is confidential. The information within this document may not be reproduced or otherwise disseminated without approval of uniQure biopharma B.V.

Sponsor Information

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EMERGENCY CONTACT INFORMATION

Any SAE, as well as any AE qualifying for special notification, occurring in a subject **from the first visit until the end of trial participation** must be reported on the paper SAE report form.

In the event of a serious adverse event (SAE) or an adverse event (AE) qualifying for special notification, the Investigator must complete the Clinical Trial Serious Adverse Events Form in the paper SAE report from.

The Investigator will ensure that the form is completed and **faxed or scanned/e-mailed to** CCI **within 24 hours** of becoming aware of the event meeting the protocol definition for an SAE or an AE qualifying for special notification. The paper SAE form and all other relevant documents supporting the reported SAE must be faxed or scanned/e-mailed to:

CCI
24 hour Hotline: PPD
Fax: PPD
E-mail: PPD

More details can be found in Section 13 of this protocol.

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List of Abbreviations

Abbreviation	Definition
AASLD	American Association for the Study of Liver Diseases
AAV	Adeno-Associated Virus
AAV2	Adeno-Associated Viral vector serotype 2
AAV5	Adeno-Associated Viral vector serotype 5
AAV8	Adeno-Associated Viral vector serotype 8C
ADR	Adverse Drug Reaction
AE	Adverse Event
AIP	Acute Intermittent Porphyria
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
aPTT	activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATMP	Advanced Therapy Medicinal Product
cDNA	complementary Deoxyribonucleic Acid
CHMP	Committee for Medicinal Products for Human Use
C_{max}	Maximum concentration
COVID-19	Coronavirus Disease 2019
CRP	C-Reactive Protein
CSR	Clinical Study Report
CTL	Cytotoxic T Lymphocyte
DIBD	Development International Birth Date
DIC	Disseminated Intravascular Coagulation
DNA	Deoxyribonucleic Acid

Abbreviation	Definition
DSUR	Development Safety Update Report
eCRF	electronic Case Report Form
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
FIX	coagulation Factor IX
γGT	gamma-Glutamyl Transpeptidase
gc	genome copies
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBeAg	Hepatitis B extracellular Antigen
HBsAg	Hepatitis B surface Antigen
HBV DNA	Hepatitis B Virus Deoxyribonucleic Acid
HCV RNA	Hepatitis C Virus Ribonucleic Acid
HD-Ad	Helper Dependent Adenovirus
hFIX	human coagulation Factor IX
hFVIII	human coagulation Factor VIII
HIV	Human Immunodeficiency Virus
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1β	Interleukin-1beta

Abbreviation	Definition
IL-2	Interleukin-2
IL-6	Interleukin-6
IMP	Investigational Medicinal Product
IND	Investigational New Drug
INFγ	Interferon gamma
INR	International Normalized Ratio
IRB	Institutional Review Board
IU	International Unit
MCP-1	Monocyte Chemotactic Protein-1
MedDRA	The Medical Dictionary for Regulatory Activities
mL	millilitre
mRNA	messenger Ribonucleic Acid
NCI	National Coordinating Investigator
nr LAM PCR	non-restrictive Linear Amplification Mediated Polymerase Chain Reaction
OTC	Over the Counter
PBGD	Porphobilinogen Deaminase
PCR	Polymerase Chain Reaction
PT	Prothrombin Time
CCI	CCI
Q-PCR	Quantitative Polymerase Chain Reaction
qs	sufficient quantity
rAAV	recombinant Adeno-Associated Virus
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

Abbreviation	Definition
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard Deviation
SDV	Source Data Verification
CCI	CCI
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
US	United States of America
VG	Vector Genomes
WHO-DD	World Health Organisation Drug Dictionary
WNL	Within Normal Limits
w/w	weight per weight

1. Sponsor's Approval of the Clinical Trial Protocol

PPD
PPD
uniQure biopharma B.V.

PPD

Signature Date

PPD
PPD
uniQure biopharma B.V.

PPD

Signature Date

2. International Coordinating Investigator's Approval of the Clinical Trial Protocol

International Coordinating Investigator

PPD

Hämophiliezentrum, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany

PPD

PPD

Date

3. Principal Investigator's Agreement to the Clinical Trial Protocol

I confirm that I have read and understood this protocol and agree to conduct this study in compliance with the Declaration of Helsinki, the International Council for Harmonisation (ICH) Guideline for GCP and applicable regulatory requirements. I confirm agreement to conduct the study in compliance with the protocol. I fully understand that any changes from the protocol constitute a deviation and will be notified to the sponsor.

Name and title in print	Signature	Date

4. Protocol Summary

4.1 Primary Objective

The primary objective of this trial is to investigate the safety of systemic administration of AAV5-hFIX, an adeno-associated viral (AAV) vector containing a codon-optimized human coagulation Factor IX (hFIX) gene, to adult patients with severe or moderately severe haemophilia B.

The primary objective will be assessed based on adverse events (AEs).

4.2 Secondary Objectives

Secondary objectives will be addressing efficacy and safety of systemic administration of AAV5-hFIX.

4.3 Trial Design

This trial has an open-label, uncontrolled, single-dose, dose-ascending design and consists of 2 cohorts. The trial will be conducted at multiple centres in multiple countries.

At study completion, subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 10 years from the IMP dosing date.

4.4 Trial Population

Male patients, aged ≥ 18 years, with severe or moderately severe haemophilia B with a severe bleeding phenotype defined as:

- Known coagulation Factor IX (FIX) deficiency with plasma FIX activity level < 1% (severe) or plasma FIX activity level ≥ 1% and ≤ 2% (moderately severe) and
- currently on prophylactic FIX replacement therapy for a history of bleeding, or
- currently on on-demand FIX replacement therapy with a current or past history of frequent bleeding (defined as 4 or more bleeding episodes in the last 12 months or chronic haemophilic arthropathy [pain, joint destruction, and loss of range of motion] in one or more joints).

Patients should have had more than 150 previous exposure days of FIX replacement therapy.

4.5 Intervention

The Investigational Medicinal Product (IMP) is a recombinant adeno-associated viral vector of serotype 5 (AAV5) containing the codon-optimized hFIX complementary deoxyribonucleic acid (cDNA) under the control of a liver-specific promoter. The IMP is identified as AAV5-hFIX. The pharmaceutical form of AAV5-hFIX is a solution for intravenous infusion.

Subjects will be allocated to one of 2 cohorts with the following planned dose levels:

- Cohort 1 (5 subjects): AAV5-hFIX 5×10^{12} gc/kg
- Cohort 2 (5 subjects): AAV5-hFIX 2×10^{13} gc/kg

The process for cohort allocation is described in Section 11.2.1. Subjects will receive the IMP on only one occasion and will thereafter be followed for 5 years with respect to safety and with respect to efficacy measured as levels of FIX, bleeding patterns and consumption of FIX replacement therapy.

4.6 Interim Analyses

Interim analyses will be performed at the request of the data monitoring committee and at the request of the sponsor to enable reporting of adequate data at selected time points during the course of the trial. Descriptive statistics alone will be used for the interim analyses.

5. Schedule of Trial Procedures

Subjects will attend a screening visit (Visit 1) at a maximum of 6 weeks prior to the anticipated dosing visit (Visit 2). When the screening visit blood sample analysis results from the local and central laboratory are available, and it is confirmed that the subject is fulfilling the trial eligibility criteria, the dosing visit can occur provided that the intra- and inter-cohort staggering principles are adhered to (see Section 10.1). Sufficient time should be allowed for IMP shipment and preparation.

In case the subject no longer fulfils the eligibility criteria at the time of the dosing visit, re-screening is allowed as detailed in Section 10.2.1.

After IMP administration, subjects will be monitored for tolerance to the IMP and detection of potential immediate AEs at the clinical trial site for 24 hours (overnight stay). Thereafter, subjects will be followed with respect to safety and efficacy parameters for 5 years (260 weeks) as follows:

- Twice weekly up to week 12
- Every 2nd week from week 12 to week 26
- Every 13th week from week 26 to week 156 (6 months to 3 years)
- Every 26th week from week 156 to week 260 (3 years to 5 years)

Clinical procedures to be performed in the period up to week 26 are listed in Table 5-1. Clinical procedures to be performed after week 26 and up to week 260 are listed in Table 5-4.

Laboratory parameters to be measured in the period up to week 26 are listed in Table 5-2. Laboratory parameters to be measured after week 26 and up to week 260 are listed in Table 5-5.

Clinical procedures to be performed in relation to IMP administration and the subsequent 24 hour observation period at the clinic (Visit 2) are listed in Table 5-3.

Adjustments to the visit location or schedule may be made to accommodate safety concerns and restrictions due to the Coronavirus Disease 2019 (COVID-19) pandemic. In all cases, subjects will be kept informed as much as possible, of changes to the study and monitoring plans that could impact them.

Table 5-1, Flow Chart for Safety and Efficacy Evaluation, Visits 1 - 21

Visit Number	**1** Screening	**2** [1] Day 1	**2** [1] Day 2	**3 to 14** (every week)	**3[b] to 14[b] (every week)**	**15 to 20** (every 2nd week)	**21**	**Additional visits** [2]
Weeks [4]	-6	0	0	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	14, 16, 18, 20, 22, 24	26	
Visit window [4]				±2 days	1-4 days after main weekly visit	±5 days	±5 days	
Informed consent	x							(x) [3]
Inclusion/exclusion criteria	x	x						(x) [3]
24 hour stay at clinic		x ---------- x						
Administration of IMP		x						
Tapering prophylactic FIX replacement therapy (see Section 11.4) [5]				X (Visits 8-14 / weeks 6-12)				
Demographics and medical history [6]	x							
History and status of bleeding	x							
History of surgery	x							
Joint status	x							(x) [2]
Subject e-diary instruction	x	x						
Subject e-diary interview (including re-instruction, if needed)		x	x	x		x	x	(x) [2]
Review of e-diary data (bleeding episodes, FIX replacement therapy)		x	x	x		x	x	(x) [2]
Registration of severity of bleeding episode(s)		x	x	x		x	x	(x) [2]
Registration of concomitant treatment other than FIX replacement therapy	x	x	x	x		x	x	(x) [2]
Blood pressure, pulse, body temperature	x	x	x	x		x	x	(x) [2]
Physical examination	x	x	x	x		x	x	(x) [2]
Height	x							
Body weight	x	x					x	(x) [2]
Blood and urine sampling (see Table 5-2)	x	x	x	x	x	x	x	(x) [2]
Adverse events	x	x	x	x		x	x	(x) [2]
CCI	x						x	

Footnotes are given on next page.

Footnotes to Table 5-1:

1) Time of procedures in relation to IMP administration is detailed in Table 5-3.
2) An additional visit can be performed at any time for the purpose of conducting one or more procedures listed in the column "Additional Visits" according to the choice of the Investigator. Hence, "(x)" refers to a procedure that can be performed, if judged relevant by the Investigator.
3) An additional visit can be performed at any time in advance of the screening visit (i.e. Visit 1), after obtaining written informed consent, for the purpose of pre-determination of subject eligibility. For this purpose a selection of the procedures listed in the column "Additional Visits" (i.e. neutralizing antibodies to AAV5, HIV, viral load, $CD4^+$, HBsAg, HBeAg, HBV DNA, HCV RNA) can be performed.
4) Each visit is scheduled in relation to date of IMP administration, not in relation to the date of its previous visit.
5) Tapering of prophylactic FIX replacement therapy may be started prior to 6 weeks after IMP administration as detailed in Section 11.4.
6) Including available information on FIX gene mutation. If FIX gene mutation information is not available and if separate informed consent for FIX gene sequencing analysis is given by the subject, a blood sample for the purpose of FIX gene sequencing analysis should be drawn (see Table 5-2).

Table 5-2, Flow Chart for Laboratory Parameters, Visits 1 – 21

Visit Number	**1** Screening	**2** Day 1	**2** Day 1	**2** Day 2	**3 to 14** (every week)	**3[b] to 14[b] (every week)**	**15 to 20** (every 2nd week)	**21**	**Additional visits** [1)]
Weeks [3)]	-6	0 Pre IMP	0 3h post IMP	0 24h post IMP	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	14, 16, 18, 20, 22, 24	26	
Visit window [3)]			±15 minutes	±60 minutes	±2 days	1-4 days after main weekly visit	±5 days	±5 days	
Local Laboratory									
FIX: one-stage aPTT or chromogenic/amidolytic assay for FIX activity for local monitoring [4)]	x	x			x	x	x	x	(x) [1)]
FIX inhibitors (Bethesda assay or Nijmegen modified Bethesda assay) for local monitoring and eligibility check [4)]	x	x			x: Visit 14			x	(x) [1)]
Liver enzymes (AST/ALT)	x	x		x	x	x	x	x	(x) [1)]
Central Laboratory									
FIX: one-stage aPTT and chromogenic/amidolytic assay for FIX activity, and FIX protein concentration	x	x			x		x	x	(x) [1)]
Anti-FIX antibodies		x						x	
FIX inhibitors (Nijmegen modified Bethesda assay)	x	x			x: Visit 14			x	(x) [1)]
FIX recovery [5)]		x							(x) [1)]
Total (IgM and IgG) antibodies to AAV5	x	x			x: Visits 3, 4, 5, 6			x	
Neutralizing antibodies to AAV5	x	x			x: Visits 3, 4, 5, 6			x	(x) [1,2)]
AAV5 capsid-specific T cells		x			x		x	x	
Sampling for vector genome detection: - Blood, urine, saliva, nasal secretions [6)] - Faeces, semen [6)]		x x		x	x x: Visits 3, 5, 8, 11, 14		x x	x x	
Inflammatory markers IL-1β, IL-2, IL-6, INFγ, MCP-1		x			x		x: Visits 15, 16, 17		
Haematology parameters [7)]	x	x			x: Visits 6, 10, 14		x: Visits 16, 18, 20	x	(x) [1)]
Coagulation parameters [8)]	x	x			x: Visits 6, 10, 14		x: Visits 16, 18, 20	x	(x) [1)]

Visit Number	**1** Screening	**2** Day 1	**2** Day 1	**2** Day 2	**3 to 14** (every week)	**3[b] to 14[b] (every week)**	**15 to 20** (every 2nd week)	**21**	**Additional visits [1)]**
Weeks [3)]	-6	0 Pre IMP	0 3h post IMP	0 24h post IMP	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	14, 16, 18, 20, 22, 24	26	
Visit window [3)]			±15 minutes	±60 minutes	±2 days	1-4 days after main weekly visit	±5 days	±5 days	
Serum chemistry parameters [9)]	x	x	x [10)]	x [10)]	x		x: Visits 16, 18, 20	x	(x) [1)]
Urine parameters [11]	x	x			x: Visits 6, 10, 14		x: Visits 16, 18, 20	x	(x) [1)]
HIV, viral load, $CD4^+$	x								(x) [1,2)]
HBsAg, HBeAg, HBV DNA and HCV RNA	x								(x) [1,2)]
FIX gene sequencing [12]	x								
Blood sample for future research [13]	x	x			x: Visits 7, 12			x	

1) An additional visit can be performed at any time for the purpose of conducting one or more procedures listed in the column "Additional Visits" according to the choice of the Investigator. Hence, "(x)" refers to a procedure that can be performed, if judged relevant by the Investigator.
2) An additional visit can be performed at any time in advance of the screening visit (i.e. Visit 1), after obtaining written informed consent, for the purpose of pre-determination of subject eligibility. For this purpose a selection of the procedures listed in the column "Additional Visits" (i.e. neutralizing antibodies to AAV5, HIV, viral load, $CD4^+$, HBsAg, HBeAg, HBV DNA, HCV RNA) can be performed.
3) Each visit and time point is scheduled in relation to date of IMP administration, not in relation to the date of its previous visit or time point.
4) Preferably, the same type of assay is used consistently for the individual subject throughout the entire trial period.
5) At Visit 2 and additionally at suspicion of FIX inhibitor or at increase in bleeding frequency, as judged by the Investigator. At each occasion a FIX challenge dose of 40 U/kg should be administered. A blood sample should be drawn just prior FIX dosing and at 30 minutes after FIX dosing.
6) Sampling applicable for the individual subject and for a specific matrix, only until 3 consecutive negative samples have been detected for the subject for that particular type of matrix. Based on the wish of the subject, faeces and semen samples can be collected at home prior to attending the visit (at the visit day or at the day before the visit day).
7) Haemoglobin, haematocrit, platelet count, red blood cells, white blood cells with differential count (all expressed in % as well as in absolute numbers).
8) aPTT and PT (INR). In addition, lupus anticoagulant and antithrombin will be measured at Visit 1.
9) Serum electrolytes (sodium, potassium), creatinine, gamma-glutamyltransferase (γGT), aspartate aminotransferase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), C-reactive protein (CRP), albumin, total bilirubin, glucose (non-fasting).
10) Blood sample only for CRP.
11) pH, protein, blood, leucocyte esterase, glucose.
12) Only if FIX gene mutation information is not available and if separate informed consent for FIX gene sequencing analysis is given by the subject. Preferably at Visit 1, but otherwise at a later time point during the subject's trial participation.
13) Only if separate informed consent is given by the subject, see also Section 12.3.11.

Table 5-3, Flow Chart for Visit 2 (Dosing Visit)

Visit	2 Day 1										2 Day 2
	Pre IMP										
Hours after IMP infusion completed		**0**	**0.5**	**1**	**2**	**3**	**4**	**6**	**8**	**12**	**24**
Inclusion/exclusion criteria	x										
Admission for overnight stay	x										
IMP infusion [1)]		x									
Subject e-diary instruction	x										
Subject e-diary interview (including re-instruction, if needed)	x										x
Review of e-diary data (bleeding episodes, FIX replacement therapy)	x										x
Registration of severity of bleeding episode(s)	x										x
Registration of concomitant treatment other than FIX replacement therapy	x										x
Blood pressure, pulse, body temperature	x		x	x	x	x	x	x	x	x	x
Body Weight	x										
Physical examination	x										x
Blood and urine sampling	x					x[2)]					x[2)]
Sampling for vector genome detection: - Blood, urine, saliva, nasal secretions - Faeces, semen [3)]	 x x										x
Adverse events	x ----------										---------- x
Completion of 24 hour stay											x

1) Start time and stop time of infusion will be recorded.
2) Blood sample only for CRP.
3) Based on the wish of the subject, the pre-dose faeces and semen samples can be collected at home prior to attending the visit (at the visit day or at the day before the visit day).

Table 5-4, Flow Chart for Safety and Efficacy Evaluation, Visits 22 – 35

Visit Number	**22**	**23**	**24, 25, 26**	**27**	**28, 29, 30**	**31**	**32**	**33**	**34**	**Additional visits [1)]**	**35 End of Trial [2)]**
Weeks [3)]	39	52 (1 year)	65, 78, 91	104 (2 years)	117, 130, 143	156 (3 years)	182	208 (4 years)	234		260 (5 years)
Visit window [3)]	±2 weeks	±2 weeks	±2 weeks	±2 weeks	±2 weeks	±2 weeks	±4 weeks	±4 weeks	±4 weeks		±4 weeks
Joint status		x		x		x		x		(x) [1)]	x
Subject e-diary interview (including re-instruction, if needed)	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Review of e-diary data [4)] (bleeding episodes, FIX replacement therapy)	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Registration of severity of bleeding episode(s)	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Registration of concomitant treatment other than FIX replacement therapy	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Blood pressure, pulse, body temperature	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Physical examination	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Body Weight	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Blood and urine sampling (see Table 5-5)	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Adverse events [4)]	x	x	x	x	x	x	x	x	x	(x) [1)]	x
CCI		x		x		x		x			x

1) An additional visit can be performed at any time for the purpose of conducting one or more procedures listed in the column "Additional Visits" according to the choice of the Investigator. Hence, "(x)" refers to a procedure that can be performed, if judged relevant by the Investigator.
2) Since this is a gene therapy trial in which the IMP is administered to human subjects for the first time, the Investigator should make all reasonable attempts to maintain the subjects in the trial to allow long-term follow-up on safety. However, should a subject wish to withdraw from the trial, the Investigator should call the subject for the End of Trial Visit (Visit 35) to have all Visit 35 procedures conducted (unless the subject has withdrawn consent to perform any further visits).
3) Each visit is scheduled in relation to date of IMP administration, not in relation to the date of its previous visit.
4) After Visit 21 and until Visit 31 the visit frequency is every 13 weeks, and after Visit 31 and until Visit 35 (End of Trial Visit) the visit frequency is every 26 weeks. In these periods, AEs and e-diary data review will be performed at least monthly, i.e. in addition to the review at each visit. Subjects who are on continuous routine FIX prophylaxis during the long-term follow-up phase of the trial are required to contact the site staff immediately in case of a bleed and/or FIX use different from their routine FIX prophylaxis, in addition to completing the questions/information requested on the e-diaries to capture all information.

Table 5-5, Flow Chart for Laboratory Parameters, Visits 22 – 35

Visit Number	**22**	**23**	**24, 25, 26**	**27**	**28, 29, 30**	**31**	**32**	**33**	**34**	**Additional visits [1)]**	**35 End of Trial**
Weeks [2)]	39	52 (1 year)	65, 78, 91	104 (2 years)	117, 130, 143	156 (3 years)	182	208 (4 years)	234		260 (5 years)
Visit window [2)]	±2 weeks	±2 weeks	±2 weeks	±2 weeks	±2 weeks	±2 weeks	±4 weeks	±4 weeks	±4 weeks		±4 weeks
Local Laboratory											
FIX: one-stage aPTT or chromogenic/amidolytic assay for FIX activity for local monitoring [3)]	x	x	x	x	x	x	x	x	x	(x) [1)]	x
FIX inhibitors (Bethesda assay or Nijmegen modified Bethesda assay) for local monitoring and eligibility check [3)]		x		x		x		x		(x) [1)]	x
Liver enzymes (AST/ALT)										(x) [1)]	
Central Laboratory											
FIX: one-stage aPTT and chromogenic/amidolytic assay for FIX activity, and FIX protein concentration	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Anti-FIX antibodies		x		x		x		x			x
FIX inhibitors (Nijmegen modified Bethesda assay)		x		x		x		x		(x) [1)]	x
FIX recovery [4)]										(x) [1)]	
Total (IgM and IgG) antibodies to AAV5		x		x		x		x			x
Neutralizing antibodies to AAV5		x		x		x		x			x
Sampling for vector genome detection:											
Blood, urine, saliva, nasal secretions [5)]	x	x	x	x	x	x	x	x	x		x
Faeces, semen [5)]	x	x	x	x	x	x	x	x	x		x
Haematology parameters [6)]	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Coagulation parameters [7)]	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Serum chemistry parameters [8)]	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Urine parameters [9)]	x	x	x	x	x	x	x	x	x	(x) [1)]	x
Blood sample for future research [10)]		x									

Footnotes are given on next page.

Footnotes to Table 5-5:

1) An additional visit can be performed at any time for the purpose of conducting one or more procedures listed in the column "Additional Visits" according to the choice of the Investigator. Hence, "(x)" refers to a procedure that can be performed, if judged relevant by the Investigator.
2) Each visit and time point is scheduled in relation to date of IMP administration, not in relation to the date of its previous visit or time point.
3) Preferably, the same type of assay is used consistently for the individual subject throughout the entire trial period.
4) At suspicion of FIX inhibitor or at increase in bleeding frequency, as judged by the Investigator. At each occasion a FIX challenge dose of 40 U/kg should be administered. A blood sample should be drawn just prior FIX dosing and at 30 minutes after FIX dosing.
5) Sampling applicable for the individual subject and for a specific matrix, only until 3 consecutive negative samples have been detected for the subject in that particular type of matrix. Based on the wish of the subject, faeces and semen samples can be collected at home prior to attending the visit (at the visit day or at the day before the visit day).
6) Haemoglobin, haematocrit, platelet count, red blood cells, white blood cells with differential count (all expressed in % as well as in absolute numbers).
7) aPTT and PT (INR).
8) Serum electrolytes (sodium, potassium), creatinine, gamma-glutamyltransferase (γGT), aspartate aminotransferase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), CRP, albumin, total bilirubin, glucose (non-fasting).
9) pH, protein, blood, leucocyte esterase, glucose.
10) Only if separate informed consent is given by the subject, see also Section 12.3.11.

6. Introduction and Rationale

6.1 Introduction to Disease Area

Congenital haemophilia B is characterized by an increased bleeding tendency due to either a partial or complete deficiency of the essential blood coagulation FIX. Haemophilia B is an X-linked, recessive condition. Approximately 1 in 25,000 live male newborns have haemophilia B (Bolton-Maggs & Pasi, 2003). The number of diagnosed haemophilia B patients globally is about 34,000 (World Federation of Haemophilia, 2018). Individuals with severe haemophilia B are usually diagnosed during the first year of life. The severity of symptoms can vary and the severe forms become apparent early in life. Bleeding is the main symptom of the disease and usually increases when the infant becomes mobile. Internal bleeding may occur anywhere and bleeding into joints is common.

Haemophilia B is caused by a variety of genetic anomalies distributed throughout the gene on the long arm of the X chromosome, with the most common being single base-pair changes that result in missense, frame shift, or nonsense mutations (Cooper et al., 2009). With a deficiency or absence of FIX, activation of coagulation factor X becomes severely impaired leading to delayed and insufficient thrombin burst for normal haemostasis. The haemostatic plug formed in these patients is fragile and easily dissolved by normal fibrinolytic activity with impaired haemostasis, prolonged bleeding episodes and re-bleeding as the consequences.

About 1/3 of individuals with haemophilia B have a severe disorder characterised by functional FIX levels that are less than 1% of normal (Kessler & Mariani, 2006). Mild and moderate haemophilia B are each observed in about 1/3 of patients (Kessler & Mariani, 2006).

FIX is synthesized as a single polypeptide chain that undergoes extensive posttranslational modifications (Furie & Furie, 1992; Mann, 1999). The liver is the primary site of FIX synthesis and hepatocytes directly secrete FIX into the plasma (Furie & Furie, 1992).

The classical laboratory findings in haemophilia B include a prolonged activated partial thromboplastin time and a normal prothrombin time (Bowyer et al., 2011).

6.2 Treatments

Haemophilia B is currently managed by intravenous injections of clotting factor in various forms such as recombinant hFIX concentrates and recombinant or plasma derived FIX concentrates, prothrombin complex concentrates or plasma, or via plasma and whole blood transfusions. Fresh frozen plasma is still the only product available for treatment of haemophilia B in several developing countries across the world. Plasma and whole blood products are less effective, since they contain lower concentrations of FIX. They also convey risks of circulation overload and heart failure but mainly carry the risk of viral contaminations. In the 1970s and 1980s, thousands of people with haemophilia around the world were infected with human immunodeficiency virus (HIV) and hepatitis C by contaminated plasma-derived products. Although safer products have emerged from this tragedy, the risk of contamination of blood products with known and unknown potentially infectious agents remains.

Because they are devoid of such risks, recombinant hFIX concentrates are widely accepted as the optimal treatment.

Current treatment for haemophilia B in the western world involves intravenous infusions of recombinant FIX concentrates at the time of a bleed ("on-demand" therapy). This is highly effective at arresting haemorrhages, but cannot prevent spontaneous bleeds or any chronic damage that ensues after a bleed. In patients with severe haemophilia B, spontaneous bleeding episodes can be dramatically reduced when plasma FIX levels are maintained continuously at, or are above 1% of normal (0.01 IU/mL) by prophylactic administration of FIX protein. However, the relatively short half-life of hFIX (approximately 19 hours according to the Summary of Product Characteristics (SPC) for BeneFIX® and approximately 25 hours according to Prescribing Information for RIXUBIS®) necessitates frequent intravenous administration of concentrates (2-3 times a week) at 40 U/kg or even higher to maintain minimum trough levels of ≥ 1%. Frequent intravenous injections are invasive, inconvenient and highly problematic for children. Since prophylaxis can reduce the risk of spontaneous bleeds and help reduce or prevent joint damage (Manco-Johnson et al., 2007; Valentino et al., 2014), it is becoming the standard of care in countries with access to adequate quantities of clotting factor concentrates. For Western countries, standard of care is to start prophylaxis at 1 year of age, or after first joint bleed.

6.3 Limitations of Current Treatment Approach: Medical Need

6.3.1 Reliance on Central Venous Access Devices

The treatment by prophylactic regular intravenous injections is not curative and is very demanding. To make regular injections easier a port-a-cath, or implantable venous access device, can be implanted under the skin, usually in the upper chest. Use of port-a cath has made prophylactic treatment easier as they provide venous access, however these devices are also associated with a risk of bacterial infections. Studies differ, but some show an infection rate as high as 50%. These infections can usually be treated with intravenous antibiotics, but sometimes the device must be removed (World Federation of Haemophilia). Also, the same source cites some studies showing a risk of clots forming at the tip of the catheter.

6.3.2 Risk of FIX Inhibitors

Approximately 3-5% of patients with severe haemophilia B develop alloantibody inhibitors that can neutralize both recombinant and plasma-derived administered FIX. These FIX inhibitors are usually immunoglobulins G (IgGs) and often appear after approximately 10 infusions of FIX concentrate (Chitlur et al., 2009; DiMichele, 2007; Warrier, 1999). However, they may appear at any time in the patient's life. Inhibitor development to FIX is considered the most severe problem in haemophilia care today as it affects the efficacy of patient treatment, increases the risk of developing joint disease, increases the cost of haemophilia care, and leads to increased morbidity.

6.3.3 Non-adherence Resulting in Increased Risk of Spontaneous Bleeds

Therapy adherence drops sharply when patients reach the teenage years and begin self-infusing and remains low thereafter (Geraghty et al., 2006). As many as 41% of patients report that they do not always take factor replacement according to their prescribed regimen.

This is of serious concern considering that as few as 1 to 2 bleeds can trigger progressive, irreversible joint disease (Gater et al., 2011).

Chronic debilitating joint disease results from recurrent bleeding into the joint, synovial membrane inflammation, hypertrophy, and, eventually, destructive arthritis. Chronic joint deformities that need to be managed by an orthopaedic specialist may occur. Furthermore, joint replacement(s) may be needed.

The overall life expectancy in severe haemophilia is approximately 72 years in patients without HIV/hepatitis C (Plug et al., 2006). In the United Kingdom Haemophilia Centre Doctors' Organisation database there were, from 1977 to 1998, a total of 10 deaths from intracranial haemorrhage in children younger than 5 years as compared with only 0.013 deaths expected in this age population in general (Darby et al., 2007).

6.3.4 Burden of the Disease

The burden of the disease is high - both for the individual patient and their families and for society. Patients may not be able to participate in certain activities (e.g. contact sports), and they encounter long-term impairments in mobility and functional status leading to absence from school or work. Issues may surface with social participation and peer integration, particularly when children are growing up. Haemophilia patients are less likely to proceed into full-time employment and occupational disability is more frequent (Fischer et al., 2013). Living with haemophilia can have a substantial effect on mental wellbeing, particularly among young people and signs of major depressive disorder are not uncommon. The economic burden for the society is significant. Haemophilia patients are accredited with requiring 2-3 times the health care resources per inhabitant in developed countries (Schramm & Berger, 2003). The described use of FIX replacement therapy varies considerably across national economies, even among the wealthiest of countries. Trends suggest that the reported FIX usage increases with increasing economic capacity and has been increasing over time. Trends also suggest that consumption of FIX has been increasing at a greater rate in high-income countries. However, approximately 70–80% of the world's haemophilia B population, primarily in the developing world, receives inadequate or no treatment because of unavailable and/or unaffordable factor concentrates (Stonebraker et al., 2011).

6.4 Scientific Rationale for Gene Therapy in Haemophilia B

Somatic gene therapy for haemophilia B offers the potential for a shift of the disease severity from severe to a moderate or mild haemophilia phenotype through continuous endogenous production of FIX after a single administration of vector, especially since a small rise in circulating FIX can substantially ameliorate the bleeding phenotype.

AAV vectors of serotypes 2 and 8 (AAV2 and AAV8), coupled to hFIX have been used in previous clinical trials of haemophilia B (Manno et al., 2003; Manno et al., 2006; Nathwani et al., 2011). The clinical experience with liver-directed AAV gene therapy includes more than 20 subjects with severe haemophilia B (Manno et al., 2003; Manno et al., 2006; Nathwani et al., 2011). The most recently published clinical trial (Nathwani et al., 2014) demonstrated that sufficient FIX expression can be achieved through intravenous infusion of AAV8-hFIX in haemophilia B subjects thus converting severe haemophilia B phenotypes to moderate/mild haemophilia B.

6.5 Rationale for AAV Serotype 5 Vector in Haemophilia B

Over the years, several clinical trials testing different AAV vectors have guided the understanding towards development of optimal configuration of an adequate gene therapy approach for haemophilia B. The overall progress of liver-directed gene transfer for haemophilia and other diseases has revealed that the risk of immune-mediated toxicity may be the most important concern and that it is multifaceted. Early clinical trials using an AAV serotype 2 elicited immune responses, hypothetically leading to loss of expression. Newer AAV serotype 8 based vectors have significantly reduced this phenomenon, but not eliminated it.

The liver-directed AAV5 vector product to be used in haemophilia B is based on the same technology as used in the ongoing clinical trial of Acute Intermittent Porphyria (AIP). The AAV5 vector is produced by uniQure, and is manufactured using uniQure's baculovirus based expression vector system, which provides a consistent and robust product quality and thereby allows for mitigating any risks linked to product quality. The lowest prevalence of neutralizing antibodies is found in the adult European population for AAV5 (3.2%) followed by AAV8 (19%) and is highest for AAV2 (59%) and AAV1 (50.5%) (Boutin et al., 2010). Furthermore, among individuals seropositive for AAV5 and AAV8, around 70-100% present low titres (Boutin et al., 2010). Since even modest neutralizing antibody titres are thought to be able to preclude efficient liver transduction (Mingozzi and High, 2011) it is of importance to limit any effects on efficacy due to pre-existing immunity to AAV. In an effort to circumvent any inhibitory effects of possible pre-existing immunity, uniQure has chosen to use the less common AAV5. However, the prevalence of neutralizing antibodies may differ depending on the region, the age and the medical history of the patients (e.g. seroconversion due to blood transfusion) and some authors have reported differing numbers (Li et al., 2012; Boutin et al., 2010; Moskalenko et al., 2000). Both aspects may contribute to optimization of the risk/benefit for liver-directed AAV based gene therapy products in haemophilia (see also Investigator's Brochure).

6.6 Investigational Medicinal Product

6.6.1 Description of IMP

The IMP is a recombinant AAV5 containing the codon-optimized hFIX cDNA under the control of a liver-specific promoter. The IMP is identified as AAV5-hFIX. The pharmaceutical form of AAV5-hFIX is a solution for intravenous infusion.

6.6.2 Non-clinical Experience

An overview of the non-clinical evaluation of AAV5-hFIX is given in Table 6-1. Further details can be found in the Investigator's Brochure.

A range of *in vivo* studies in wild-type and haemophilia B mice and in non-human primates have been performed to characterize the safety and pharmacology of AAV5-hFIX. Infusion of AAV5-hFIX to mice and non-human primates resulted in vector dose-dependent circulating levels of (human) FIX protein. To verify that AAV5-hFIX mediates expression of biologically active hFIX, and to confirm that the hFIX produced and secreted into the circulation can ameliorate the clotting defect inherent to FIX deficiency, a dose-range study has been performed in a murine model of haemophilia B (for details, see the Investigator's Brochure).

In both Rhesus and Cynomolgus macaques, injected at various doses with AAV5-hFIX the resulting hFIX expression correlated with the dose and was sustained for the duration of the 6 months follow-up (Cynomolgus macaque) and 90 days follow-up (Rhesus monkey).

None of the animals presented elevated liver enzymes levels or other signs of toxicity during the whole observation period, and after sacrifice, no abnormalities were observed in the liver. Further details are available in the Investigator's Brochure.

These non-clinical data suggest that intravenous administration of AAV5-hFIX is able to mediate sustained levels of FIX, and that such administration is not associated with any significant safety concerns.

Table 6-1, Overview of Non-clinical Safety and Pharmacology Studies and Key Findings

Study	Endpoint	Status	Key findings
Biological activity of AMT-060 assessed in C57Bl/6 mice for 28 days. (Non-GLP) (Study NR-060-11-007)	Detection of vector DNA in liver Detection of hFIX in blood Dose-response (FIX levels)	Completed	Targeting of vector to the liver. Dose dependent levels of vector DNA in liver. Dose-dependent circulating human FIX protein levels over 4 weeks period.
90-days toxicity and biodistribution study in Rhesus monkeys (non-GLP) (Study 520665 and NR-060-11-009)	Local tolerance Human FIX levels at the chosen dose Clinical chemistry and haematology Analysis of vector DNA after 3 months in selected organs Gross necropsy and histopathology	Completed	Some weight loss in the treated animals. No further signs of any toxicities / No histopathological findings. Human FIX is expressed in the liver and circulating levels of the protein are detected over the 3 months period.
180-days toxicity and biodistribution study in Cynomolgus macaques (GLP) (Study 522156 and NR-060-14-006)	Local tolerance, Effect on cytokines, Dose-response (FIX levels), Clinical chemistry and haematology, Biodistribution of vector DNA after 6 months, mRNA levels in liver, Antibodies to human FIX Gross necropsy and histopathology	Completed	Some weight loss in the treated animals. No further signs of any toxicities / No histopathological findings. At 6 months, vector DNA in liver and adrenals. Dose-dependent human FIX expression, circulating hFIX protein levels over the 6 months observation period and hFIX in the liver. One animal with significant levels of xenogeneic antibodies against human FIX. hFIX circulating levels decreased in direct relation with the antibody formation.

Study	Endpoint	Status	Key findings
180-days toxicity and biodistribution study in C57Bl/6 mice (GLP) (Study 522657 and NR-060-13-006)	Local tolerance, Acute immune reactions, Dose-response (FIX levels), Clinical chemistry and haematology, Biodistribution of vector DNA and persistence over 6 months, mRNA in liver Gross necropsy and histopathology	In-life and histopathology completed. Biodistribution ongoing	No weight loss or signs of any toxicities / No histopathological findings. Pending Q-PCR analysis. Dose-dependent human FIX expression, circulating hFIX protein levels over the 6 months observation period. No immune response against human FIX. Prednisone has a negative effect on the levels of circulating hFIX.
Germ line transmission / Breeding study in C57Bl/6 mice (GLP) (Study 496402)	Biodistribution of vector DNA to reproductive organs and to foetuses via paternal line.	In-life completed, tissues analysis ongoing	No specific observations so far. Pending Q-PCR analysis.
Biological activity of AAV5-hFIX in mFIX deficient (haemophilia B) mice (Non-GLP) (Study NR-060-13-007)	Dose-response (FIX levels) Evaluation of the biological activity of the expressed human FIX in a murine FIX deficient haemophilia B mouse model	Completed	Direct correlation *in vivo* between circulating human FIX protein levels and human FIX activity
In vitro biological activity (potency) in Huh-7 cells. (non-GLP) (Study NR-060-12-001)	Assay to determine the biological activity of AMT-060 *in vitro* in human hepatocyte-derived cell line	Completed	Direct correlation *in vitro* between the expressed hFIX protein levels and the measured FIX activity
Integration site analysis on liver tissue obtained from C57B/l6 mice and non-human primates (*Macaca fascicularis*) injected with AAV5-hFIX vector (AMT-060) (Non-GLP) (Study NR-060-14-007)	Liver samples from studies 522657 and 522156 (4 animals of each dose group for mice, all animals for non-human primates) sacrificed at Day 180. Integration site analysis by nrLAM-PCR and high throughput sequencing.	Completed	No preferred integration in the genes MECOM (MDS1-EVI1), in LMO2 or in HMGA2. In mice CIS were found in active genes such as Alb, Ttc39c, Esp38, Lrrc4c.

Study	Endpoint	Status	Key findings
Validation of Q-PCR method (GLP) (Study 314899)	Validation of the Q-PCR method to determine vector DNA levels in tissues, body fluids and excreta from cynomolgus monkeys and assess biodistribution and shedding	Completed	Method validated in a representative panel of matrices
Validation of Q-PCR method (GLP) (Study 20041333)	Validation of the Q-PCR method to determine vector DNA levels in tissues and body fluids from C57Bl/6 mice and assess the biodistribution and PK of the vector DNA	Completed	Method validated in a representative panel of matrices
Evaluation of the long-term expression of hFIX in new-born, juvenile and adult mice after single injection of AAV5-hFIX (Non-GLP) (Study NR-060-14-008)	Local tolerance, Body weight (gain), Circulating human FIX levels, Gross necropsy and histopathology	Ongoing	No weight loss or signs of any toxicity so far. Circulating hFIX levels are stable but depend on the age and liver size at the moment of injection.

GLP: Good Laboratory Practice

6.6.3 Clinical Experience with Similar Products

Emerging AAV Gene Therapy for Haemophilia A and B
Patients with haemophilia are ideal candidates for gene therapy, as even a small increase in protein production may lead to significantly decreased bleeding diathesis. Over the years several clinical trials testing different vectors have guided the understanding toward development of optimal configuration of an adequate gene therapy approach for haemophilia B (Cancio et al., 2013), see Table 6-2.

Table 6-2, Clinical Trials with Gene Therapy for Haemophilia A and B

Reference	Year	Vector	Gene delivered	Subjects (n)	Administration	Outcome/comments
Roth et al.	2001	Plasmid DNA	hFVIII	6	Laparoscopic injection of ex vivo genetically altered fibroblasts in the omentum	Safe and well tolerated. No benefit long term
Powell et al.	2003	γ–retroviral	hFVIII	13	Intravenous infusion	Safe and well tolerated. Short lived circulating FVIII
White & Monahan	2005	HD-Ad vector	hFVIII	1	Intravenous infusion	Severe hepatotoxicity and DIC. No detectable FVIII expression
Manno et al.	2003	AAV2	hFIX	8	Intramuscular injection	Safe and well tolerated. First parenteral administration of rAAV in humans. Circulating FIX levels < 1.5%
Manno et al.	2006	AAV2	hFIX	7	Hepatic artery infusion	Transient transaminase elevation. FIX expression detected but only transiently with loss of expression concurrent with transaminase elevation
Nathwani et al.	2014	AAV8	hFIX	10	Intravenous infusion	Mild, transient transaminase elevation in 4 out of 6 high dose subjects. Stable FIX expression > 1% for up to 48 months. Effect on transient transaminase elevation on FIX levels unclear

The first 2 AAV gene therapy clinical trials for haemophilia B consisted of an AAV2 encoding FIX. The route of administration was via intramuscular injection and hepatic artery infusion, respectively (Manno et al., 2003; Manno et al., 2006).

The hepatic artery approach produced the most promising results. However, some of the findings from this recombinant AAV administration procedure were the observation of transient, apparently Cytotoxic T Lymphocyte (CTL) mediated liver enzyme perturbations that peaked 2-4 weeks after vector infusion. An inverse correlation between pre-existing

neutralizing antibody titres towards the vector, and the ability to achieve peak FIX expression was observed. These results led to the consideration that the selection of AAV vector serotype may be an important differentiator for both safety and efficacy of gene therapy.

The third AAV gene therapy clinical trial used an alternate AAV serotype (i.e. AAV8), which seems to be less frequently found in humans as compared to AAV2 (Calcedo et al., 2009), less efficiently transduces antigen-presenting cells as compared to AAV2 (Vandenberghe et al., 2006) and has increased liver tropism as compared to AAV2 (Nathwani et al., 2007), which enabled peripheral vein infusion of the vector. Results on the first 6 subjects from the clinical trial were published in 2011 (Nathwani et al., 2011). The 6 adult subjects, all suffering from severe haemophilia B, were intravenously infused via a peripheral vein with 1×10^{11} gc/kg (low dose), 6×10^{11} gc/kg (medium dose), or 2×10^{12} gc/kg (high dose) of AAV8-hFIX (Nathwani et al., 2011). An additional 4 subjects have subsequently been treated with the high dose (Nathwani et al., 2014). The population in the clinical trial by Nathwani comprised severe haemophilia patients both on on-demand treatment and on continuous prophylaxis. The clinical trial demonstrated that stable FIX expression can be achieved through intravenous infusion of AAV8-hFIX in haemophilia B patients thus potentially converting severe haemophilia B phenotypes to mild haemophilia B (see Table 6-3).

Table 6-3, AAV8 Vector Approach in Haemophilia B Patients

	Vector		**FIX expression level**	**S-ALT levels**		
No	Serotype	Dose (VG/kg)	Steady state FIX level	Peak (IU/L)	Time	Steroids
1	AAV8	$2x10^{11}$	2.17%	WNL	-	No
2	AAV8	$2x10^{11}$	1.4%	WNL	-	No
3	AAV8	$6x10^{11}$	2.86%	WNL	-	No
4	AAV8	$6x10^{11}$	2.17%	WNL	-	No
5	AAV8	$2x10^{12}$	3.56%	202	8 weeks	Prednisolone
6	AAV8	$2x10^{12}$	7.21%	36	9 weeks	Prednisolone
7	AAV8	$2x10^{12}$	5.00%	43	7 weeks	Prednisolone
8	AAV8	$2x10^{12}$	6.67%	WNL	-	No
9	AAV8	$2x10^{12}$	5.23%	WNL	-	No
10	AAV8	$2x10^{12}$	2.89%	64	9 weeks	Prednisolone

Adapted from Nathwani et al., 2014.

The intravenous administration of AAV8-hFIX did not cause any unexpected or serious adverse events (SAEs). A single intravenous infusion of vector in all 10 subjects with severe haemophilia B resulted in a dose-dependent increase in circulating FIX to a level that was 1 to 6% of the normal value over a median period of 3.2 years, with observation ongoing. In the high-dose group, a consistent increase in the FIX level to a mean (± standard deviation [SD]) of 5.1 ± 1.7% was observed in all 6 subjects, which resulted in a reduction of more than 90% in both bleeding episodes and the use of prophylactic FIX concentrate (Nathwani et al., 2014). Of the 7 subjects who were receiving FIX concentrate prophylactically at the time of vector infusion, 4 were able to discontinue therapy without having spontaneous bleeding. Vector DNA was found in excretions and secretions, but was cleared around 6 weeks after administration. A transient increase in the mean alanine aminotransferase level occurred between week 7 and week 10 in 4 of the 6 subjects in the high-dose group but resolved over a median of 5 days (range, 2 to 35) after prednisolone treatment. None of the 10 subjects have developed inhibitors to FIX.

The elevation of serum aminotransferase levels appeared to be different to what had occurred in the previous liver-directed AAV clinical trial, by manifesting itself later following vector administration. The causal relationship and the significance of the liver enzyme perturbations currently remain unclear. It has been speculated that the liver enzyme increase is related to a specific T cell reactivity (CTL). Two subjects at the lower dose level of AAV8 presented with high levels of CTL reactivity to capsid as observed in peripheral blood mononuclear cells without concurrent elevation of liver enzyme levels.

The clinical results published by Nathwani (Nathwani et al., 2011 and Nathwani et al., 2014) has invigorated the field and, currently, 3 ongoing clinical trials that also apply AAV based liver-directed gene therapy approaches for the treatment of haemophilia B are registered on www.clinicaltrials.gov (NCT01620801; NCT00979238; NCT01687608).

Optimized AAV gene therapy for treatment of haemophilia B
AAV5-hFIX has been designed to further optimize the risk/benefit balance for haemophilia B by balancing adequate potency and longevity of expression with any potential risk, see Section 6.5.

A clinical trial with a similar AAV5 vector produced by uniQure for the treatment of AIP, displayed promising safety data at similar dose levels as planned to be used for the treatment of haemophilia B (Table 6-4). Eight subjects with AIP were treated with 4 escalating dose levels with 2 subjects dosed in each cohort. The maximum dose was 2 x 10^{13} gc/kg. Liver biopsies were performed in 6 out of 8 subjects.

Table 6-4, AAV5 Vector Approach in Acute Intermittent Porphyria

	Vector		PBGD expression level		S-ALT levels		
No	Serotype	Dose (gc/kg)	Liver biopsy	Detectable PBGD DNA	Peak (IU/L)	Time	Steroids
1	AAV5	5 x 10^{11}	Yes	+	WNL	6 months	No
2	AAV5	5 x 10^{11}	Yes	++	WNL	6 months	No
3	AAV5	2 x 10^{12}	Yes	+	WNL	6 months	No
4	AAV5	2 x 10^{12}	Yes	++	WNL	6 months	No
5	AAV5	6 x 10^{12}	No	N/A	WNL	6 months	No
6	AAV5	6 x 10^{12}	Yes	+++	WNL	6 months	No
7	AAV5	2 x 10^{13}	Yes	++	WNL	6 months	No
8	AAV5	2 x 10^{13}	No	N/A	WNL	6 months	No

A 6 months interim analysis of the clinical safety data has been performed. For a period of 6 months after administration of the liver-directed AAV gene therapy based on a similar AAV5 approach as AAV-FIX, no safety concerns have been identified. In particular, no liver enzyme perturbations have been noted at any dose level and no rescue administration of corticosteroids has been administered.

As described earlier, the liver-directed AAV5 vector product to be used in this trial is based on the same technology as the product used in an ongoing clinical trial in AIP. uniQure has licensed the liver gene expression cassette for hFIX used in the clinical trial published by Nathwani et al. (Nathwani, 2011). In this clinical trial the gene cassette demonstrated the

ability to achieve adequate and prolonged expression of human FIX protein levels (Nathwani et al., 2014).

In summary, although AAV5-FIX has not yet been tested in subjects with haemophilia B, a significant number of subjects have already been exposed in clinical trials to similar products containing relevant components of AAV5-hFIX, such as the capsid and the hFIX gene cassette, although not used together.

Furthermore, significant amount of published human safety information and experience has been established with AAV gene therapy approaches in general and for liver-directed AAV gene therapy in haemophilia B in particular (AAV2 and AAV8).

6.7 Risk/Benefit Considerations

Gene therapy for haemophilia B offers the potential benefit for a shift of the disease severity from severe to a moderate or mild haemophilia phenotype or complete amelioration of the haemophilic state through continuous endogenous production of FIX after a single administration of vector.

The identified risks are considered low and manageable and not affecting the risk/benefit balance in an unfavourable way. uniQure's optimized AAV5 approach has the potential to further limit the risks currently associated with AAV gene therapy approaches.

The following risks have to be considered:

Risk of infusion-related toxicity
To date no infusion related toxicities have been reported in clinical trials with liver-directed AAV gene therapy approaches and at dose levels comparable with dose levels planned to be applied in this first human AAV5-hFIX clinical trial. This potential risk is being addressed by administering the infusion of AAV5-FIX during hospitalisation combined with in-hospital post-administration surveillance of up to 24 hours.

Risk of immune mediated neutralisation of the AAV5 gene transfection
- Pre-existing neutralizing antibodies against the AAV5 capsid potentially result in significantly reduced bioavailability of AAV5-hFIX, and hence significantly reduced efficacy. Excluding subjects with pre-existing neutralizing antibodies against the AAV5 capsid minimizes this potential risk. Subjects who in spite of this precaution do not achieve adequate expression will be able to continue their current regimen with FIX replacement therapy.
- Subjects administered the vector will develop antibodies against the viral capsid proteins and these antibodies are likely to persist. This is unlikely to cause any symptoms or lack of expression of FIX, but prevents any future administration or second dose of the same vector, since these antibodies may neutralize newly infused vector capsids. The rationale for the starting dose level is addressing this risk by increasing the likelihood of achieving a therapeutic benefit also at the lowest dose level.

Risk of immune mediated liver toxicity
Intravenous administration of a liver-directed AAV vector might lead to transaminase elevation. Previous clinical trials have shown that potential elevations in liver enzymes react promptly with normalization after administration of glucocorticoids. Subjects in the trial will be monitored twice weekly during the first 12 weeks after infusion of AAV5-hFIX for the occurrence of transaminase elevations, which may warrant the initiation of a corticosteroid treatment protocol.

Risks to third parties and the environment related to shedding via body fluids
The AAV vector will distribute systemically and small amounts of vector DNA have been observed in previous non-clinical and clinical studies in secreta and excreta, especially saliva and urine, for a limited period of time. The vector is non-pathogenic and cannot replicate. Therefore, the risk for third parties such as family and health care personnel is considered marginal. Due to the incapacity of replication, the non-infectious nature of the shed DNA and the negligible amounts shed, the risk to the environment can be considered negligible. No specific containment or protection measures are deemed necessary.

Risk of vector DNA integration into the host genome
AAV vector genomes remain mainly episomal after transduction of the target cells with minimal integration into the mammalian genome and with no association with oncogenicity. Integration site analysis was carried out by non-restrictive Linear Amplification Mediated Polymerase Chain Reaction (nr LAM PCR) and subsequent high throughput sequencing on DNA extracted from the livers of both mouse and cynomolgus macaques after administration of AAV5-hFIX at various doses. There was no preferred integration in genes known to mediate malignant transformation or clonal dominance. Both episomal (concatemeric) and integrated forms of AAV5-hFIX were retrieved, but the sequences were present almost exclusively as non-integrated episomal forms. The retrieved integrants were randomly distributed throughout the host genome. No specific clustering was seen in cynomolgus macaque genome, while some level of clustering around active genes was seen in the mouse. There were no signs of *in vivo* clone selection in the animals. The risk of oncogenicity will be assessed clinically during the duration of the trial, see Section 10.2.5.

Risk of germ-line transmission of vector DNA
The risk of germ line transmission is considered negligible for AAV-based vectors due to the marginal integration level of the vector DNA into the host genome. Any potential risk is addressed by requiring the use of a condom during the trial in the period from administration of the investigational drug until the AAV5 vector has been cleared from semen, as evidenced by negative analysis results for AAV5 vector for at least 3 consecutively collected semen samples.

Risk of off-target expression of the transgene
The vector will distribute systemically to all tissues thereby potentially infecting other cells than liver cells resulting in off-target gene expression. This risk is addressed by the use of a liver-specific promoter in the gene cassette. In other clinical trials using similar vector approaches, no AEs have been reported that could be related to potential off-target expression.

Risk of inhibitor formation to FIX expressed from the transgene
There is a risk of inhibitor/antibodies development against the expressed FIX protein. No FIX inhibitor formation was seen in any of the previous clinical trials where subjects were exposed to hFIX gene transfer and where the expressed levels of FIX were measurable (Manno et al., 2003; Manno et al., 2006; Nathwani et al., 2011 and 2014). This risk is being addressed by using the same hFIX gene cassette as was used in the Nathwani study which has shown to result in successful and adequate FIX expression levels without development of FIX inhibitors. In addition, subjects will be selected on the basis of a low risk of FIX inhibitor development by choosing subjects with more than 150 exposure days to a FIX product as well as omitting subjects with a previous FIX inhibitor. Subjects will be regularly monitored for FIX inhibitor development.

Risk of breakthrough bleeding
The scope of the liver-directed AAV gene therapy approach is to establish a stable and durable expression of FIX and to convert a severe haemophilia phenotype to a mild phenotype. Previous clinical trials with similar AAV vectors and the identical gene cassette have shown that stable and year-long FIX expression can be achieved (Nathwani et al., 2014). In order to reduce the risk of breakthrough bleedings during the early course of the clinical trial, a tapering approach is implemented regarding reduction in prophylactic treatment and including close monitoring of plasma FIX concentrations before stop of prophylactic therapy.

Furthermore, subjects are monitored closely for 5 years after administration of IMP and the need for renewed prophylactic therapy will be clinically assessed by the treating physician according to local standard of care for haemophilia subjects.

6.8 Accommodations Due to the COVID-19 Pandemic

In the first quarter of 2020, a pandemic was announced for COVID 19, which is caused by the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The pandemic impacted the conduct of clinical trials due to quarantines, site closures, travel limitations, diversion of resources and general interruptions in study related procedures, leading to protocol deviations. This study protocol includes contingency measures to manage disruptions due to COVID 19 illness and/or public health control measures; see Section 10.1.1 for details on measures related to adjustments to visit location/method and schedule. In all cases, subjects will be kept informed as much as possible, of changes to the study and monitoring plans that could impact them. The impacts of these implemented contingency measures on the outcomes of this study, including any protocol deviations that ultimately result from COVID-19 illness and/or COVID-19 public health control measures will be discussed in the Clinical Study Report (CSR).

The decision to test a subject in the study for COVID-19 should be based on the site's current guidelines and at the discretion of the Investigator. If a subject participating in the study is identified as a person under investigation for possible COVID-19 infection, the Investigator should notify appropriate authorities as per site's regulations and notify uniQure's Medical Directors. As co-infections can occur, the site should consider all subjects for COVID-19 virus testing regardless of whether another respiratory pathogen is found.

If a subject is confirmed positive for COVID-19 at any time during the trial, the medical care, isolation, and management should be according to national, local, institutional, and public health guidelines.

7. Purpose of the Trial

The trial is designed to investigate the safety and efficacy of AAV5-hFIX in patients with haemophilia B. In addition, the trial is intended to provide data to enable decisions regarding the further development of AAV5-hFIX in haemophilia B.

8. Trial Objectives and Endpoints

8.1 Objectives

8.1.1 Primary Objective

To investigate the safety of systemic administration of AAV5-hFIX, an adeno-associated viral vector containing a codon-optimized hFIX gene, to adult patients with severe or moderately severe haemophilia B

8.1.2 Secondary Objectives

Secondary objectives will be addressing the efficacy and safety of systemic administration of AAV5-hFIX to adult patients with severe or moderately severe haemophilia B:

Efficacy Objectives

- To investigate the effect of AAV5-hFIX on FIX activity level
- To investigate the effect of AAV5-hFIX on the use of FIX replacement therapy
- To investigate the effect of AAV5-hFIX on bleeding episodes
- To investigate the effect of AAV5-hFIX on CCI parameters

Safety Objectives

- To monitor shedding of the vector in various body matrices (i.e. fluids/excretions)
- To monitor the immune responses against AAV5 capsid proteins in response to AAV5-hFIX
- To monitor for immune responses against FIX protein after administration of AAV5-hFIX
- To investigate the effect of AAV5-hFIX on inflammatory markers

8.2 Endpoints

8.2.1 Primary Endpoint

Adverse events

8.2.2 Secondary Endpoints

Secondary endpoints will be addressing efficacy and safety:

Confirmatory Secondary Efficacy Endpoint

- FIX-replacement-therapy-free FIX activity[1, 2]

Supportive Efficacy Endpoints

- Bleeding rate
- Total consumption of FIX replacement therapy
- CCI scores

[1] Definition of FIX-replacement-therapy-free FIX activity: FIX activity measured any time from 72 hours after latest FIX replacement therapy administration and until next administration of FIX replacement therapy, if any
[2] FIX activity will be measured with both the one-stage aPTT assay and the amidolytic/chromogenic assay.

Safety Endpoints

- Vector DNA in semen, blood, saliva, nasal secretions, urine and faeces
- Neutralizing antibodies to AAV5
- Total (IgM and IgG) antibodies to AAV5
- AAV5 capsid-specific T cells
- Antibodies to FIX
- FIX inhibitors
- Inflammatory markers: IL-1β, IL-2, IL-6, INFγ, MCP-1

9. Trial Population

9.1 Population

Subjects will be adult patients with haemophilia B and will be recruited from haemophilia centres in multiple countries. A minimum of 10 subjects will be enrolled: 5 subjects into each of 2 dose cohorts. The Sponsor may approve additional enrolment of up to 2 subjects in Cohort 2. The numbers of enrolled subjects projected for each clinical trial site will be specified in other document(s) such as the Clinical Trial Application forms.

The subjects' either severe or moderately severe haemophilia B should be of a severe bleeding phenotype as defined in inclusion criterion no. 3 below.

Recruitment will be controlled such that a maximum of 2 subjects with moderately severe haemophilia B phenotype are enrolled per cohort.

9.2 Inclusion Criteria

1. Male
2. Age ≥ 18 years
3. Patients with congenital haemophilia B classified as one of the following:
   - Known severe FIX deficiency with plasma FIX activity level < 1% and a severe bleeding phenotype defined by one of the following:
     - Currently on prophylactic FIX replacement therapy for a history of bleeding
     - Currently on on-demand FIX replacement therapy with a current or past history of frequent bleeding defined as 4 or more bleeding episodes in the last 12 months or chronic haemophilic arthropathy (pain, joint destruction, and loss of range of motion) in one or more joints
   - Known moderately severe FIX deficiency with plasma FIX activity level between ≥ 1% and ≤ 2% and a severe bleeding phenotype defined by one of the following:
     - Currently on prophylactic FIX replacement therapy for a history of bleeding
     - Currently on on-demand FIX replacement therapy with a current or past history of frequent bleeding defined as 4 or more bleeding episodes in the last 12 months or chronic haemophilic arthropathy (pain, joint destruction, and loss of range of motion) in one or more joints
4. More than 150 previous exposure days[3] of treatment with FIX protein.
5. Acceptance to use a condom during sexual intercourse in the period from IMP administration until AAV5 has been cleared from semen, as evidenced by the central laboratory from negative analysis results for at least 3 consecutively collected semen samples (this criterion is applicable also for subjects who are surgically sterilized)
6. Following receipt of verbal and written information about the trial, the subject has provided signed informed consent before any trial related activity is carried out.

[3] Regarding exposure days: Prophylaxis, prevention, on-demand and treatment during surgery counts as exposure days. If it is not possible to count the actual number of exposure days based on the medical record, the Investigator should make a written statement with an estimate based on e.g. subject's age, treatment frequency, medical history, discussion with previous doctors, transfer notes and other relevant information. This statement should be filed in the subject's medical record.

9.3 Exclusion Criteria

1. History of positive FIX inhibitor test
2. Positive FIX inhibitors test at Screening (measured by the local laboratory)
3. Neutralizing antibodies against AAV5 at Screening (measured by the central laboratory)
4. Screening laboratory values (measured by the central laboratory):
   - ALT > 2 times upper normal limit
   - AST > 2 times upper normal limit
   - total bilirubin > 2 times upper normal limit
   - ALP > 2 times upper normal limit
   - creatinine > 1.5 times upper normal limit
5. Positive HIV serological test at Screening, not controlled with anti-viral therapy as shown by $CD4^+$ counts ≤ 200 per µL or by a viral load of > 200 copies per mL (measured by the central laboratory)
6. Active infection with Hepatitis B or C virus as reflected by Hepatitis B Surface Antigen (HBsAg), Hepatitis B extracellular Antigen (HBeAg), Hepatitis B Virus deoxyribonucleic acid (HBV DNA) or Hepatitis C Virus ribonucleic acid (HCV RNA) positivity, respectively, at Screening (measured by the central laboratory).
7. History of Hepatitis B or C exposure, currently controlled by antiviral therapy
8. Any coagulation disorder other than haemophilia B
9. Thrombocytopenia, defined as a platelet count below 50×10^9/L, at Screening (measured by the central laboratory)
10. Body mass index < 16 or ≥ 35 kg/m^2
11. Planned surgery for the initial 6 months after IMP administration in this trial
12. Previous arterial or venous thrombotic event (e.g. acute myocardial infarction, cerebrovascular disease and venous thrombosis)
13. Active severe infection or any other significant concurrent, uncontrolled medical condition including, but not limited to, renal, hepatic, haematological, gastrointestinal, endocrine, pulmonary, neurological, cerebral or psychiatric disease, alcoholism, drug dependency or any other psychological disorder evaluated by the Investigator to interfere with adherence to the protocol procedures or with the degree of tolerance to the IMP
14. Known significant medical condition including disseminated intravascular coagulation, fibrinolysis and liver fibrosis which, in the opinion of the Investigator, may confound, contraindicate or limit the interpretation of either safety or efficacy data
15. Known history of an allergic reaction or anaphylaxis to FIX products
16. Known uncontrolled allergic conditions or allergy/hypersensitivity to any component of the IMP excipients
17. Previous gene therapy treatment
18. Receipt of an experimental agent within 60 days prior to Visit 1
19. Current participation or anticipated participation within one year after IMP administration in this trial in any other interventional clinical trial involving drugs or devices.

9.4 Withdrawal of Individual Subjects

Since this is a gene therapy trial in which the IMP is administered to human subjects for the first time, the Investigator should make all reasonable attempts to maintain the subjects in the trial after IMP administration to allow long-term follow-up of safety. However, a subject can withdraw from the trial, at any time, if it is the wish of the subject or in the best interest of the

subject as per the Investigator's assessment. The standard of care of the subject will not be affected in any way.

Should a subject wish to withdraw from the trial at his own request or based on a decision of the Investigator the Investigator should call the subject for the End of Trial Visit (Visit 35) to have all Visit 35 procedures conducted, unless the subject has withdrawn consent to perform any further visits.

9.4.1 Replacement of Individual Subjects Withdrawn

If a subject withdraws from the trial prior to IMP administration or withdraws from the trial within the first 12 weeks after IMP administration, the subject will be replaced. Possible additionally enrolled subjects number 6 and 7 in Cohort 2 will not be replaced.

10. Trial Design

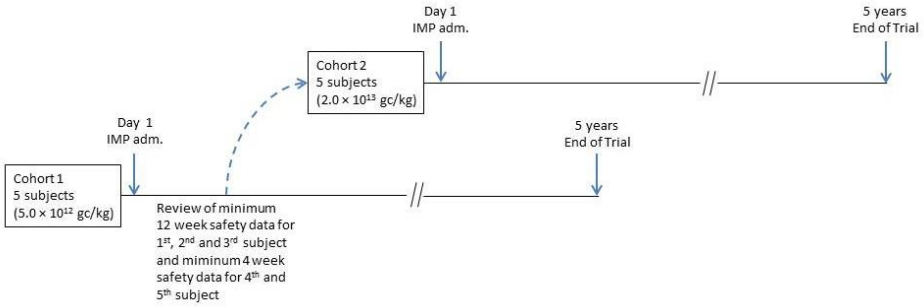
10.1 Overall Trial Design

This trial has an open-label, uncontrolled, single-dose, dose-ascending design and consists of 2 cohorts, each of a minimum of 5 subjects. Due to the low prevalence of severe and moderately severe haemophilia B patients with a severe bleeding phenotype, the trial will be conducted at multiple centres in multiple countries. Since this is a first-in-man single dose escalation trial with a high-risk medicinal product, an adequate information communication system between the sites will be in place (details will be described in the Trial Procedures Manual).

Subjects fulfilling the eligibility criteria will be allocated to either Cohort 1 or Cohort 2 as described in Section 11.2.1. Each subject will receive a single dose of IMP (AAV5-hFIX) and will thereafter be followed for 5 years with respect to safety and with respect to efficacy measured as levels of FIX, bleeding patterns, and consumption of FIX replacement therapy.

Cohort 2 will be initiated based on the recommendation of the data monitoring committee after review of safety data from Cohort 1. These data will include 12-week follow-up for the first 3 subjects dosed as well as 4 weeks follow-up for the remaining 2 subjects in Cohort 1 (see Figure 10-1). If transaminase elevations requiring corticosteroid treatment have occurred in one or more of the first 3 subjects in Cohort 1, the data monitoring committee may recommend to extend the follow-up period, before sponsor decision to initiate Cohort 2 is taken. Inter-cohort stopping criteria are described in Section 19.2.1.

Figure 10-1, Inter-cohort Staggering Interval



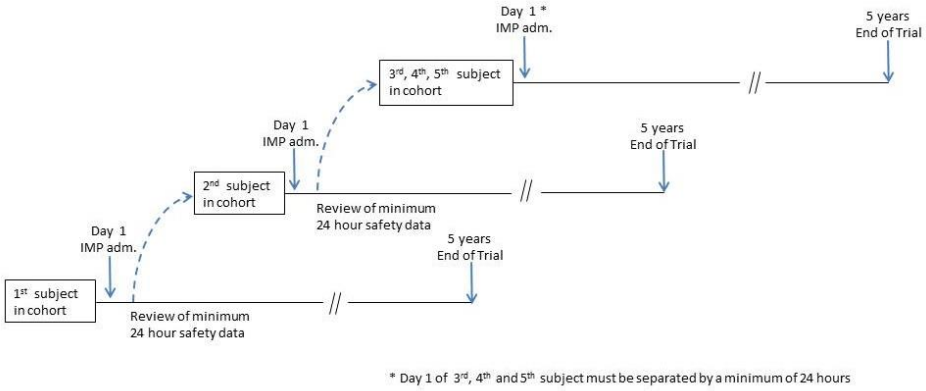
Within each cohort, there will be an observation period between IMP administration to the 1st and 2nd subject and between IMP administration to the 2nd subject and subsequent subjects (Figure 10-2). Intra-cohort stopping criteria are described in Section 19.2.1.

- After the 1st subject has been dosed in a cohort, the data monitoring committee will evaluate available safety data collected during a period of minimum 24 hours after IMP administration to this first subject. The data monitoring committee will recommend if and when dosing of the 2nd subject can be initiated
- After the 2nd subject has been dosed in a cohort, the data monitoring committee will evaluate safety data collected during a period of minimum 24 hours after IMP

administration to this second subject. The data monitoring committee will recommend if and when dosing of the subsequent 3 subjects can be initiated
- The dosing of the 3rd, 4th and 5th, and possibly 6th and 7th subject within a cohort must be separated by a minimum of 24 hours to ensure observation of any acute reactions.

Details on an adequate information communication system with and between the sites to ensure the timings of subject dosing within a cohort are adhered to will be described in the Trial Procedures Manual.

Figure 10-2, Intra-cohort Staggering Interval



After IMP administration, subjects will remain at the clinical trial site for 24 hours for monitoring of tolerance to IMP and for detection of potential immediate AEs. Hereafter, subjects will be followed with respect to safety and efficacy parameters for 5 years (260 weeks):
- Twice weekly up to week 12
- Every 2nd week from weeks 12 to 26
- Every 13th week from weeks 26 to 156 (6 months to 3 years)
- Every 26th week from weeks 156 to 260 (3 years to 5 years).

Subjects on prophylactic FIX replacement therapy will be tapered of their prophylactic FIX replacement therapy in the period from Visit 8 to Visit 14 (weeks 6-12). If the Investigator determines that FIX replacement therapy should be re-initiated, based on FIX levels or for other reasons, prophylactic FIX replacement therapy may be tapered again at a later time point. In relation to the withholding of FIX replacement therapy, additional visits may be required for the purpose of additional monitoring of FIX activity levels (see Table 5-2). For further details, please see Section 11.4.

During the entire trial period, blood samples will be drawn regularly for measurements of FIX activity and FIX protein concentration in plasma. For subjects on prophylactic FIX replacement therapy, attempts should be made to allow blood sampling at time points where FIX activity and FIX protein concentration are expected to be at lowest justifiable levels. For further information, please see Section 12.1.1.

10.1.1 Considerations Due to the COVID-19 Pandemic

Due to the COVID-19 pandemic, adjustments to the visit location/method or schedule may be made to accommodate safety concerns and restrictions experienced by individual subjects and sites. In all cases, subjects will be kept informed, via the site staff, as much as possible, of changes to the study and monitoring plans that could impact them.

Discontinuation of subjects from the study post-treatment with AMT-060 is not considered to be in the best interest of the subject, due to the irreversible nature of the IMP. Wherever possible, every effort is to be made to have the subject visit the clinic for the study visits according to schedule. Should a clinic visit not be possible, options that may be considered include site nurses travelling to a subject's home, local laboratory use, or home nursing services (for certain visits, if pre-approved by the Investigator and uniQure). These options, if used, will be supplemented with a phone call or telemedicine/telehealth safety follow-up call. A (temporary) transfer of a subject to an alternate clinical trial sites may also be considered in order to continue on-site visits, only if this does not pose undue burden to the subject and/or "new" site.

In some instances, it may not be possible to conduct any type of visit at all. Where none of the above options are feasible, subject visits may be moved beyond the maximum visit window permitted. Such delays will be assessed on a case by case basis. Until visits are rescheduled, supplemental phone calls or telemedicine/telehealth contact between Investigator/study staff and subject are to be arranged.

Supplemental phone calls or telemedicine/telehealth safety follow-up calls will be used to confirm the subject's status and wellbeing. At these calls, safety information should be gathered (i.e. AEs, concomitant medication use), subjects should be asked about any new unreported bleeds or FIX consumption and confirm their use of the e-diary (as applicable), and there should be continued discussions with the subject on the importance of a healthy liver. These discussions will be documented in the source documents.

All deviations from the study protocol are to be documented, with rationale. If a protocol deviation is due to the COVID-19 pandemic, this will be noted.

10.2 Trial Periods

10.2.1 Screening Visit (Visit 1)

The screening visit, Visit 1, will take place at a maximum of 6 weeks prior to the anticipated IMP dosing visit (Visit 2).

Before any trial related activity takes place, signed informed consent must be obtained from the subject (see Section 16.2).

All subjects undergoing screening procedures will be allocated a subject ID number and will be listed in a log. The subject ID number will consist of a 2-digit number for the clinical trial site followed by a consecutive 2-digit number for the subject.

If pre-determination of subject eligibility is desired by the Investigator in advance of the conduct of the full screening visit (Visit 1), an additional visit can be performed during which a selection of laboratory parameters can be measured (see Table 5-2, additional visits). When pre-determination of subject eligibility is established and the subject continues into the trial, a full screening visit (Visit 1) needs to be conducted (see Table 5-1 and Table 5-2, Visit 1), however if the timing of the additional visit is within 2 weeks of Visit 1 there is no need to repeat the laboratory parameters previously measured.

Eligibility according to the trial inclusion and exclusion criteria will be evaluated at Screening and during the period up to IMP administration at Visit 2.

During the period up to Visit 2, laboratory results from blood and urine samples taken at Screening (either at Visit 1 or at an additional visit for pre-determination of subject eligibility) will be provided by the central laboratory and the local laboratory and will be decisive for the evaluation of a number of exclusion criteria (see Section 9.3).

At visits where the CCI is to be completed, the subject will be asked to complete this prior to any other visit procedure is initiated. For Visit 1, this applies, however, after informed consent has been obtained.

Subjects qualifying as screen failures (i.e. subjects who fail to meet the inclusion criteria and/or fail to not meet any of the exclusion criteria during screening) may be re-evaluated for participation in the trial once. The definition of screen failure also applies to subjects who experience a bleeding episode, surgery or another event after Visit 1 that warrants a delay of Visit 2. In this situation, where the screening period exceeds the maximum of 6 weeks re-screening will require a renewed informed consent to be obtained from the subject, a new electronic Case Report Form (eCRF) should be started and the subject should be allocated a new subject ID number.

10.2.2 Dosing Visit (Visit 2)

Visit 2 should be planned to take place as an overnight stay at the clinic and should take place within a maximum of 6 weeks after Visit 1.

At Visit 2 the trial inclusion and exclusion criteria assessed at Visit 1 should be re-assessed, including any laboratory analysis results (see Section 9.2 and 9.3).

Pre-IMP samples for laboratory analysis will be collected. For details, please see Table 5-2 (under “Visit 2, Day 1, pre-IMP”).

Hereafter, IMP should be administered according to the procedures described in Section 11.2.2 and in the IMP Handling Manual.

The clinical assessments and laboratory sampling procedures to be followed in relation to IMP administration are given in Table 5-3.

The subject may leave the clinic after all assessments at 24 hours after IMP administration have been performed.

10.2.3 Follow-up Period (Visits 3 – 35)

The procedures to be followed at Visits 3 – 35 are given in Table 5-1 and Table 5-4 and the laboratory samples to be collected are given in Table 5-2 and Table 5-5.

At Visits 21 (26 weeks), 23 (1 year), 27 (2 years), 31 (3 years), 33 (4 years) and 35 (5 years) the subject should complete the CCI [redacted]. At these visits, the subject will be asked to complete the CCI [redacted] prior to any other visit procedure is initiated.

Options for how visits in the follow-up period may occur to accommodate safety concerns and restrictions due to COVID-19 are described in Section 10.1.1.

10.2.4 Additional Visits

The subject may be called in for additional visits, at the discretion of the Investigator. The subject may also contact the clinical trial site for an additional visit.

An extra visit may include additional assessments, as deemed necessary by the Investigator, such as blood sampling for FIX activity (see Section 11.4), blood sampling for FIX inhibitors (Section 12.3.10), repetition of blood sampling due to erroneous results (Section 12.4), pre-determination of subject eligibility (see Section 10.2.1), conduct of measurements that were missed at the previous visit or repetition of instructions to the subject regarding subject diaries (Section 10.3.1).

10.2.5 Follow-Up after End of Trial

The individual subjects will be followed for 5 years after administration of IMP. This is in line with the guideline on follow-up of subjects administered with a gene therapy medicinal product (EMEA/CHMP/GTWP/60436/2007).

At study completion, subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 10 years from the IMP dosing date.

10.3 Use of Subject e-Diary

From Visit 1 (after completion of all screening assessments) and throughout the entire trial participation period, subjects will be requested to document use of FIX replacement therapy and record information on bleeding episodes in a dedicated electronic subject diary (e-diary).

10.3.1 Training in the Use of Subject e-Diary

At Visit 1, subjects will be trained in the use of the e-diary system by the Investigator or study nurse. Furthermore, the subject will be provided with a written instruction. Training will be repeated at a minimum at Visit 2 as well as at any subsequent visit as judged necessary by the Investigator or study nurse.

At each visit after Visit 1, subjects will be interviewed with respect to problems, if any, related to e-diary data entry. Furthermore, the Investigator and study nurse will have access to the data entered in e-diaries. At each subject visit, e-diary data will be reviewed. Based on

interviews and e-diary data review the Investigator and/or study nurse will evaluate if training needs to be repeated. In case e-diary training is repeated, this will be reported in the subject's medical record.

After Visit 21 and until Visit 31 the visit frequency is every 13 weeks, and after Visit 31 and until Visit 35 (End of Trial Visit) the visit frequency is every 26 weeks. In these periods, e-diary data review will be performed at least monthly, i.e. in addition to the review at each visit (as described in Section 12.1.2 and 12.1.3). The Investigator or study nurse will review the data entered in e-diaries and if judged needed based on this review, the Investigator or study nurse will call in the subject for an additional visit for the purpose of a repeated training in the use of the subject e-diary. Subjects who are on continuous routine FIX prophylaxis during the long-term follow-up phase of the trial are required to contact the site staff immediately in case of a bleed and/or FIX use different from their routine FIX prophylaxis, in addition to completing the questions/information requested on the e-diaries to capture all information.

10.4 Rationale for Trial Design

A dose ascending design is chosen to determine the safety and tolerability of 2 dose levels.

As a secondary objective, the trial design supports the scope to explore the maximum achievable bioactivity of AAV5-hFIX as assessed by FIX-replacement-therapy-free FIX activity levels at the 2 dose levels.

The objective for the first cohort is to explore whether 5×10^{12} gc/kg can achieve therapeutically relevant bioactivity levels of >2% of FIX-replacement-therapy-free FIX activity without safety concerns.

The objective for the second cohort is to explore whether 2×10^{13} gc/kg can achieve an expression level of FIX in the range of 5-10% of FIX-replacement-therapy-free FIX activity without safety concerns.

The design is based upon the non-clinical data (see Section 6.6.2) and the available data from prior clinical experience with similar AAV-vector based gene therapy products (see Section 6.6.3) that supports that safe and therapeutically relevant expression levels of FIX may be anticipated with AAV5-hFIX at one or both of the 2 dose levels (see also the dose rationale in Section 10.5).

To support subject safety, the trial is designed with 2 consecutive dose arms with ascending doses and data monitoring committee review before start of dosing in the next cohort. Subjects are therefore not randomly assigned to the 2 arms, which introduces a risk of selection bias. Part of this risk will be mitigated by strict adherence to inclusion and exclusion criteria and in addition only 2 subjects with moderately severe haemophilia will be allowed in each cohort thereby preventing that the moderately severe subjects are allocated to the lowest dose level only.

Due to the nature of the disease in question it is not ethical to perform a placebo-controlled trial and no relevant active comparators exist. Based on this the trial is designed as an open-label and uncontrolled trial.

10.5 Dose Rationale

The dose selection is based on a combination of safety and efficacy data from non-clinical studies and from prior in-human experience with similar AAV based products (see Section 6.6.3).

The non-clinical safety for AAV5-hFIX has been examined in rodents and non-human primates using single doses up to 2.3×10^{14} and 0.9×10^{14} gc/kg, respectively, without showing any adverse reaction and the No Observed Adverse Effect Level was set in both species as the highest tested dose. These data establish an adequate safety margin for the highest proposed dose level of 2×10^{13} gc/kg.

The non-clinical pharmacology studies suggest that hFIX expression levels of 5-10% of normal human level can be achieved and that a dose relationship can be expected. In rhesus macaques 5-10% expression level was achieved with a dose of 5×10^{12} gc/kg. In cynomolgus macaques the expression levels achieved with similar dose levels was lower, however clear dose response from 5×10^{11} to 1×10^{14} was established. Despite observed differences between rhesus and cynomolgus monkeys in FIX expression levels at similar dose levels, the levels of hFIX expressed are dose dependent in all tested species and it is anticipated that this also applies to humans. The study in cynomolgus macaques indicates that a dose of 5×10^{11} gc/kg may be sub-therapeutic in humans and the proposed Minimum Anticipated Biological Effect Level is therefore 5×10^{12} gc/kg body weight. Despite anticipated differences between non-human primates and humans, a starting dose level at 5×10^{12} gc/kg is justified in order to minimize the risk of exposing haemophilia B patients to sub--therapeutic dose levels in the first cohort.

The combination of the AAV5 capsid and the hFIX gene cassette of AAV5-hFIX has not yet been administered to humans in clinical trials. However, the serotype 5 capsid and the gene cassette have separately been used in previous clinical trials without safety concerns (see Section 6.6.3).

In conclusion, the available non-clinical and clinical data support the safety of both the starting dose and the top dose. Furthermore, the data support the expectation that safe and therapeutically relevant expression levels of FIX may be anticipated with AAV5-hFIX at one or both of the 2 dose levels.

10.6 Rationale for Proposed Intra- and Inter-cohort Staggering Intervals

The specification of the intra- and inter-cohort staggering intervals is based on a combination of generic safety surveillance principles applicable for a first in human clinical trial, and the absence of acute and sub-acute AEs in the preclinical studies with AAV5-hFIX and the timing, frequency and severity of expected AEs based upon clinical studies with similar liver-directed AAV products that contain the AAV vector serotype and gene cassette components of AAV5-hFIX.

The data monitoring committee will evaluate available safety data collected during the initial 24 hours after IMP administration prior to dosing of the second subject in a cohort and prior to the decision to dose the subsequent subjects in a cohort in order to adequately assess the risk for subsequent subjects of any acute AEs observed. The dosing of the 3rd, 4th and 5th, and possibly 6th and 7th subject will be separated with at least 24 hours. The intra – and inter-cohort stopping criteria are described in Section 19.2.1.

The data monitoring committee will evaluate all available safety data collected during the period of a minimum of 12 weeks after IMP administration in the first 3 subjects of Cohort 1 and for a minimum of 4 weeks after IMP administration in subjects 4 and 5.

- The requirement to follow 3 subjects for 12 weeks is a precautionary measure to secure that the low risk or even absence of liver enzyme perturbations requiring corticosteroid treatment demonstrated at the highest dose level with AAV5-Porphobilinogen Deaminase (PBGD) will be reproduced with the lowest dose level of AAV5-FIX
- The rationale for the 12 weeks in the first 3 subjects is based on the data from the AAV8-hFIX clinical trial described in Section 6.6.3, where mild liver enzyme perturbations occurred during a window of 7-9 weeks after intravenous administration
- The rationale for the 4 weeks is based on the consideration to allow the data monitoring committee to adequately assess potential unexpected and significant sub-acute AEs.

10.7 Rationale for Stopping Prophylactic FIX Replacement Therapy

In the clinical trial with AAV8-hFIX, measurements of FIX activity showed an initial burst lasting up to 4 weeks after administration of AAV8-hFIX (Nathwani et al., 2011; Nathwani et al., 2014). In the clinical trial, it was possible to stop or reduce prophylactic FIX replacement therapy in 4 of 7 subjects once evidence of sustained FIX activity at 3-5% was achieved. The time point for stopping or reducing prophylactic FIX replacement therapy varied between 6 and 15 weeks.

In this trial, a similar attempt to stop or reduce prophylactic FIX replacement therapy has been implemented, following an algorithm based on FIX activity measurements, as outlined in Section 11.4.

11. Trial Treatment

11.1 Investigational Medicinal Product (IMP)

11.1.1 Description of IMP

The IMP is a recombinant AAV5 containing the codon-optimized hFIX cDNA under the control of a liver-specific promoter. The IMP is identified as AAV5-hFIX.

The pharmaceutical form of AAV5-hFIX is solution for intravenous infusion.

AAV5-hFIX will be supplied in 2 mL glass vials filled with a total volume of 1.2 mL IMP to guarantee at least 1 extractable mL of IMP. The components of the AAV5-hFIX drug product are given in Table 11-1.

CCI [redacted]

For further information, please see the Investigator's Brochure.

11.1.2 Packaging, Labelling and Storage of the IMP

Packaging and labelling of the IMP for all clinical trial sites will be coordinated by uniQure. IMP used in this trial will be prepared and labelled according to GMP, GCP and local regulatory requirements.

CCI [redacted]

For further information, including information on labelling of vials and boxes, please see the IMP Handling Manual.

11.1.3 IMP Accountability, Traceability and Disposition

The Investigator must ensure that a designated person (e.g. pharmacist) receives the IMP from the sponsor. Upon receipt of the IMP, the Investigator or designee must maintain adequate records of all IMP received, dispensed, administered and returned or destroyed

using a drug accountability log. This drug accountability log must be available for inspection at any time.

In addition, an in-clinic traceability record is maintained for the prepared IMP infusion bag to collect information about infusion bag details (e.g. expiry date/time), IMP administration details (e.g. date of infusion and start/stop time of infusion), waste disposal details (e.g. type of materials disposed), as well as details by whom the infusion bag dispensed, collected and returned.

The Investigator or designee must ensure that the IMP is maintained under required storage conditions in a secure area with restricted access. The IMP may only be used in accordance with this approved protocol and must not be used for any other purpose. The IMP may only be used for subjects who have provided written informed consent to participate in this trial and meet all trial inclusion criteria and none of the exclusion criteria.

Return/Destruction authorization forms must be completed by the Investigator or designee each time IMP is returned or destroyed, but only after written authorization is provided by the sponsor. The drug accountability log will be reviewed by the trial monitor. Upon completion of review, the trial monitor will provide the site with the written authorization by the sponsor to destroy the IMP or return it to the sponsor.

Disposal of hazardous material (e.g. syringes, needles, etc.) must be conform applicable laws and regulations. See the IMP Handling Manual for further instructions on accountability and disposition of IMP.

11.1.4 Preparation of the IMP for Intravenous Infusion

The IMP will be supplied in 2 mL vials containing 1.2 mL of IMP as a solution for intravenous infusion. Based on the assigned cohort and the subject's body weight the volume of IMP to be administered will be calculated. The solution for intravenous infusion will be drawn from the vials to prepare the final total volume. Detailed preparation instructions will be provided in the IMP Handling Manual and must be followed.

11.2 IMP Administration

11.2.1 Dose Levels and Allocation to Dosing

Subjects will be allocated to the cohorts with the following planned dose levels based on the chronological order the subjects complete the screening visit (Visit 1):

- Cohort 1 (5 subjects): AAV5-hFIX 5×10^{12} gc/kg
- Cohort 2 (5 subjects): AAV5-hFIX 2×10^{13} gc/kg

Dosing will take place once (at Visit 2). No other medication will be provided in the trial.

11.2.2 Method of IMP Administration

AAV5-hFIX will be administered as an intravenous infusion with a common intravenous infusion device. The required volume of AAV5-hFIX will depend on the cohort and the subject's body weight.

Detailed IMP administration instructions and instructions for the recording of the IMP administration details will be provided in the IMP Handling Manual and must be followed.

11.2.3 Recommendation of Treatment in Case of Increased Liver Transaminases

Background Information
The combination of the AAV5 capsid and the hFIX gene cassette of AAV5-hFIX has not previously been administered to humans in clinical trials. However, the capsid serotype 5 and the gene cassette have separately been used in previous clinical trials without safety concerns (see Section 6.6.3).

Two gene therapy clinical trials targeting the liver with other AAV serotypes have been published (Nathwani et al., 2011 and 2014; Manno et al., 2006), and in both of the studies a transient transaminase elevation was observed in some subjects (see Section 6.6.3 for further details).

Although the risk of encountering significant liver enzyme perturbations with AAV5-hFIX is considered low, it cannot be ruled out that short-term treatment with corticosteroids may be required.

In case the Investigator judges that treatment with corticosteroids may be required, it is recommended to follow the scheme below. However, the Investigator - based on local clinical practice and medical judgement - may decide to deviate from the outlined recommendation.

Recommended approach and treatment
Treatment is recommended to be instituted if, in the absence of alternative aetiology, the ALT level increase is greater than 1.5 – 2-fold the baseline level (Day 1, pre-IMP).

Based on previous clinical trials (Nathwani et al., 2011 and 2014; Manno et al., 2006) where liver enzymes elevations were observed, corticosteroids could be used for a maximum of 6 months (see Table 11-2). The Investigator is recommended to initiate treatment with prednisolone as soon as possible after detection of ALT elevation. A combined immunosuppressant regimen or the use of other products can also be considered in case of prednisolone treatment failure or contraindication.

Table 11-2, Use of Prednisolone for the Treatment of Transaminase Elevation

Timeline	Prednisolone dose (mg/day)
Week 1	60
Week 2	40
Week 3	30
Week 4	30
Maintenance until ALT returns to baseline level (Day 1, pre-IMP)	20
After pre-IMP level has been reached	Reduce daily dose with 5 mg/week

11.3 Concomitant Medication/Therapy

11.3.1 Allowed Medication

Subjects will continue their usual FIX replacement therapy (on-demand and prophylactic), as applicable. However, for subjects on prophylactic FIX replacement therapy, prophylaxis will be tapered when endogenous production of FIX is anticipated to have reached adequate levels (see Section 11.4).

11.3.2 Disallowed Medication

The following treatments will not be allowed during trial participation:

- Treatment in another interventional clinical trial involving drugs or devices within 1 year from IMP administration in this trial
- Another gene therapy treatment and/or participation in another gene therapy clinical trial

Apart from the above listed treatments, no protocol restrictions will apply with respect to concomitant treatment.

11.3.3 Reporting of Concomitant Medication/Therapy

Concomitant medication/therapy is any medication/therapy being continued by the subject at trial entry and any new medication received during the trial.

At every visit the Investigator or a qualified designee will ask the subject about concomitant medication. The Investigator should record the use of all medication (including over the counter [OTC] medication, vitamin and/or mineral supplements, homeopathic remedies and herbal preparations) used and changes in the use of medication, with the exception of FIX replacement therapy for which only the prescribed regimen should be recorded. Information on actual FIX replacement therapy taken will be reported separately by the subject in an e-diary (see Sections 12.1.2 and 12.1.3). The Investigator should also record other concomitant treatments/therapy, e.g. physiotherapy.

The following information will be recorded:

- Drug name (generic name preferred)
- Indication
- Dosing regimen (dose, unit, route, frequency)
- Route of administration
- Start date (if started ≥ 3 months prior to Visit 1, then this can be stated instead of recording the specific start date)
- Stop date (or ongoing, if ongoing at end trial participation).

11.4 Tapering and Subsequent Withholding of Prophylactic FIX Replacement Therapy

In the period of 6 to 12 weeks after IMP administration (i.e. Visits 8-14, both visits included) subjects who are on prophylactic FIX replacement therapy will be tapered of their prophylactic therapy. The tapering of prophylactic therapy should only be initiated if the FIX activity level is ≥ 2.0% at the previous 2 consecutive visits (as measured by the local laboratory).

The exact date on when the tapering of prophylactic FIX replacement therapy is initiated within the 6 to 12-week period will be decided by the Investigator. The tapering of prophylactic FIX replacement therapy may be started prior to 6 weeks after IMP administration at the discretion of the Investigator (in consultation with the subject), but only after consultation with the Sponsor's Medical Officer.

The Investigator will record the date of initiating the tapering of prophylactic FIX replacement therapy and the reason for the date chosen.

Tapering of prophylactic FIX replacement therapy
The tapering of prophylactic FIX replacement therapy will take place in 3 steps (over 2 weeks).

1. During the first week, the subject will receive 50% of his usual prophylactic dose per infusion. The number of infusions per week should not be changed.
2. During the second week, the subject will receive 25% of his usual prophylactic dose per infusion. Again, the number of infusions per week should not be changed. The decision to go from 50% to 25% of the usual prophylactic dose will be made based on an individual assessment of the subject by the Investigator, and will include the requirement that the subject is able to maintain a FIX activity level ≥ 2.0% (as measured by the local laboratory).
3. From the third week onwards, no further prophylactic dosing should be administered except in situations of e.g. major surgery. The decision to go from 25% of the usual prophylactic dose to no prophylactic dose will be made based on an individual assessment of the subject by the Investigator, and will include the requirement that the subject is able to maintain a FIX activity level ≥ 2.0% (as measured by the local laboratory).

Alternatively, the frequency of FIX prophylaxis infusions may be decreased, or the prophylactic FIX replacement therapy may be stopped in one step at the discretion of the Investigator (in consultation with the subject).

Actions during the first period of withholding prophylactic FIX replacement therapy (variable timespan)
The decision to continue to withhold the prophylactic FIX replacement therapy will be made based on an individual assessment of the subject by the Investigator, and will include the requirement to document that the subject is able to maintain a FIX activity level ≥ 2.0% (as measured by the local laboratory) for at least 2 consecutive measurements.

The choice for the time points of the 2 consecutive measurements is guided by an algorithm that is described in Table 11-3, and is used to

- Decide to either to continue to withhold the prophylactic FIX replacement therapy, or to re-start prophylactic FIX replacement therapy
- Determine the time point for next FIX activity level assessment

If per the algorithm, the next time point would not be on a business day, it may be moved to a business day at the discretion of the Investigator.

Table 11-3, Actions During the First Period of Withholding Prophylactic FIX Replacement Therapy

FIX activity level (%) [a]	Action (re-measurement or re-start of prophylactic therapy) [b]
≥ 8.0	Continue to withhold prophylactic FIX replacement therapy and assess FIX activity level again in 4-5 days
5.0-7.9	Continue to withhold prophylactic FIX replacement therapy and asses FIX activity level again in 3-4 days
3.0-4.9	Continue to withhold prophylactic FIX replacement therapy and asses FIX activity level again in 2-3 days
2.0-2.9	Continue to withhold prophylactic FIX replacement therapy and asses FIX activity level again in 1-2 days
< 2.0	Re-start prophylactic FIX replacement therapy according to local practices

[a] FIX activity will be measured by a central laboratory using the one-stage aPTT assay for FIX and the chromogenic/amidolytic assay for FIX, and by the local laboratory at each clinical trial site using the one-stage aPTT assay for FIX or the chromogenic/amidolytic assay for FIX. Analysis results from the local laboratory will be regarded as decisive for actions related to withholding FIX therapy.

[b] In case the required time for next FIX activity measurement (according to Table 11-3) does not correspond with time for next clinic visit (according to Table 5-2), then one or more additional visits should be scheduled.

Second period of withholding prophylactic FIX replacement therapy (4 weeks timespan)
If FIX activity levels are ≥ 2.0% at both of the 2 measurements during the first period of withholding prophylactic FIX replacement therapy, the withholding can be continued and the subject is followed with weekly FIX activity measurements for an additional 4 weeks. In case the required time points for these 4 weekly FIX activity measurements do not correspond with time points for the next clinic visits (according to Table 5-2), then one or more additional visits should be scheduled.

After the second period of confirming FIX activity levels has been completed, the FIX activity levels will be assessed at the per protocol scheduled visits, as listed in Table 5-1 (Visits 1 - 21) and Table 5-4 (Visits 22 - 31).

Re-starting prophylactic FIX replacement therapy after (or during) tapering
It is the intention that FIX replacement therapy is tapered for all subjects, provided the requirement of FIX activity level continuously being ≥ 2.0% is met. Prophylactic FIX replacement therapy is re-started should the FIX activity level be < 2.0%. In addition, the Investigator may at any time decide for medical reasons to re-start prophylactic FIX replacement therapy.

Subsequent re-initiating of tapering the prophylactic FIX replacement therapy may be done if clinically justified. The same requirements for minimum FIX activity level and the following up on the FIX activity levels as described above (i.e. first and second period of withholding prophylactic FIX replacement therapy) should be adhered to.

In case of any bleeding episode (spontaneous, traumatic, recurrent, related to surgical intervention, or other), on-demand FIX replacement therapy should be administered at the discretion of the Investigator, according to local practice.

12. Assessments

12.1 Efficacy Assessments

12.1.1 FIX Activity for Efficacy Evaluation

Blood samples for FIX activity will be collected at all visits, including at the additional visits as required during or after the tapering of prophylactic FIX replacement therapy as described in Section 11.4. At Visit 2, blood sampling will take place prior to IMP administration.

Throughout the entire trial period (from Visit 1 and onwards) where a subject is on prophylactic FIX replacement therapy, it will be the aim to draw blood samples at time points where FIX activity is expected to be at the lowest levels. To the extent visit windows allow, the Investigator and/or study nurse will collaborate with the subject to schedule visits to take place on days where prophylactic FIX replacement therapy is planned to be administered. At these visits, blood sampling will then take place just prior to administration of prophylactic FIX replacement therapy, which will then be administered at the clinic instead of at home.

The following FIX activity assays will be applied at a central laboratory:
- one-stage activated Partial Thromboplastin Time (aPTT) assay
- chromogenic/amidolytic assay

12.1.2 Bleeding Episodes

From Visit 1 and throughout the entire trial participation period, subjects will be asked to record information on bleeding episodes in an e-diary. Regarding training of subjects in the use of the e-diary, please see Section 10.3.1.

The subject e-diary will include questions regarding each bleeding episode with respect to:
- Date and time of onset of bleed
- Date and time of stop of bleed
- Location of bleed
- Circumstances of bleed (spontaneous, traumatic, minor surgery), see definitions at the end of this section for further details)
- FIX replacement treatment of bleed (medication, dose, date and time of administration)
- Other therapy (pain relieving medication, compression, ice)
- Evaluation of effect of treatment (excellent, good, moderate, none), see definitions at the end of this section for further details.

At each visit, the Investigator will review e-diary entries on bleeding episodes and the subject's medical/hospital records on bleeding episodes (if any). After Visit 21, e-diary data review will be performed at least monthly in addition to the review at each visit. E-diary entries and medical/hospital records on bleeding episodes will be considered source data. The Investigator will record his/her assessment of the severity of the bleed (see definitions below).

In case of bleeding episodes fulfilling a seriousness criterion (i.e. the bleeding episode is a SAE), information on the bleeding episodes is not required to be entered in the e-diary but in the medical/hospital records only. In those cases, the Investigator will also record his/her evaluation of effect of treatment of bleed (excellent, good, moderate, none), if applicable, see definitions at the end of this section for further details. Safety reporting requirements for disease-related bleeding episodes are described in Section 13.8.

Definitions

Minor surgery
Any invasive operative procedure for which only the skin, the mucous membranes or superficial connective tissue is manipulated. Examples include implanting pumps or ports in subcutaneous tissue, skin biopsies and simple dental procedures.

Circumstances of bleed
Only spontaneous bleeds, traumatic bleeds and minor surgery are collected in the e-diary and eCRF. Data on bleeds related to major surgery requiring in-patient hospitalization are collected via SAE forms. Classification of re-bleed will be performed by the trial statistician at the time of analysis according to the following criteria: A re-bleed is defined as a worsening of symptoms in the same location after an initial period of improvement, either on treatment or within 72 hours after stopping treatment. If a bleed occurs in the same location later than 72 hours after stopping, the bleed is considered a new bleed.

Subject's evaluation of effect of treatment of bleed
Excellent: Abrupt pain relief and/or equivocal improvement in objective signs of bleeding within approximately 8 hours of a single infusion
Good: Definite pain relief and/or improvement in signs of bleeding with approximately 8 hours of one infusion, but possibly requiring more than one infusion for complete resolution
Moderate: Probable or slight beneficial effect approximately 8 hours after the first infusion; usually requiring more than one infusion
None: No improvement, or worsening of symptoms

Investigator's evaluation of severity of bleed
Mild/moderate bleeds:
- Uncomplicated joint bleeds
- Muscular bleeds without compartment syndrome
- Mucosal bleeds
- Subcutaneous bleeds

Severe bleeds:
- Complicated joint bleeds
- All intracranial, retroperitoneal, iliopsoas or neck bleeds
- Muscle bleeds with compartment syndrome
- Bleeds associated with a decrease in haemoglobin levels of more than 2 g/dL

Investigator's evaluation of effect of treatment of bleed in case of bleeding episodes being a SAE

Excellent: Abrupt pain relief and/or equivocal improvement in objective signs of bleeding within approximately 8 hours of a single infusion
Good: Definite pain relief and/or improvement in signs of bleeding with approximately 8 hours of one infusion, but possibly requiring more than one infusion for complete resolution
Moderate: Probable or slight beneficial effect approximately 8 hours after the first infusion; usually requiring more than one infusion
None: No improvement, or worsening of symptoms

12.1.3 Prophylactic FIX Replacement Therapy

From Visit 1 and throughout the entire trial participation period, subjects will be asked to document use of FIX replacement therapy in an e-diary. Regarding training of subjects in the use of the e-diary, please see Section 10.3.1.

The subject e-diary will include questions with respect to

- Date and time of administration
- Drug name
- Dose

At each visit, the Investigator will review e-diary entries on prophylactic FIX replacement therapy and the subject's medical/hospital records on prophylactic FIX replacement therapy (if any). After Visit 21, e-diary data review will be performed at least monthly in addition to the review at each visit. E-diary entries and medical/hospital records on prophylactic FIX replacement therapy will be considered source data. Subjects who are on continuous routine FIX prophylaxis during the long-term follow-up phase of the trial are required to contact the site staff immediately in case of a bleed and/or FIX use different from their routine FIX prophylaxis, in addition to completing the questions/information requested on the e-diaries to capture all information.

CCI [redacted].

12.2 Safety Assessments

12.2.1 Adverse Events

Information on AEs will be reported at each visit. After Visit 21, AEs will be assessed at least monthly.

Details regarding definitions of AEs, definitions of AE assessments, how to obtain information on AEs, how to report AEs and how to follow-up on AEs are described in Section 13.

AEs and SAEs that the Investigator becomes aware of should continue to be reported to CCI [redacted] following the completion of the study for a period of up to 10 years following gene therapy dosing, until/unless the subject is enrolled in the extension protocol CT-AMT-060-04.

12.2.2 Physical Examination

A physical examination will be performed at all visits. At Visit 2, the physical examination will be performed both prior to IMP administration and at 24 hours after IMP administration. The physical examination will include general appearance and bedside examination of the following body systems: Lymph nodes, eyes and ears, mouth and throat, lungs, abdomen, extremities, musculoskeletal system, neurological system, cardiovascular system and skin.

The evaluation of each body system will be recorded as “normal” or “abnormal”. Abnormalities will also be recorded.

Any abnormality judged by the Investigator as a clinically relevant worsening since Visit 1 should be reported as an AE.

12.2.3 Blood Pressure, Pulse and Body Temperature

Blood pressure, pulse and body temperature will be measured at all visits. At Visit 2 (dosing visit with overnight stay) blood pressure, pulse and body temperature will be measured prior to IMP administration and at the following approximate time points after IMP administration: 0.5, 1, 2, 3, 4, 6, 8, 12 and at 24 hours.

Before measurement of blood pressure and pulse the subject should rest for at least 5 minutes. For the individual subject, all measurements should be performed while the subject is in the same position (i.e. sitting or lying) throughout the trial.

Body temperature should be measured using the same method (e.g. an ear thermometer) for the individual subject throughout the trial.

Any abnormality judged by the Investigator as a clinically relevant worsening since Visit 1 should be reported as an AE.

12.2.4 Serum Chemistry, Haematology, Coagulation and Urine Parameters for Central Lab

Table 12-1 provides the safety laboratory parameters to be measured:

Visits at which samples should be drawn as well as a specification of the parameters to be measured are given in Table 12-2 and Table 12-3.

Table 12-1, Safety Laboratory Parameters

Serum Chemistry	Serum electrolytes (sodium, potassium), creatinine, ɣGT, AST, ALT, ALP, CRP, albumin, total bilirubin, glucose (non-fasting)
Haematology	Haemoglobin, haematocrit, platelet count, red blood cells, white blood cells with differential count (band forms, segment forms, neutrophils, eosinophils, basophils, monocytes, lymphocytes) (all expressed in % as well as in absolute numbers)
Coagulation	aPTT, PT (or INR [International Normalized Ratio]), lupus anticoagulant, antithrombin
Urine parameters	pH, protein, blood, leucocyte esterase, glucose

Table 12-2, Sampling for Serum Chemistry

Visit number	Week in relation to IMP administration	Time point at Visit	Parameter to be measured
1	Maximum 6 weeks prior to anticipated IMP administration	Any time at visit	All parameters
2	-	Prior to IMP administration	All parameters
2	-	3 and 24 hours after IMP administration	CRP only
3 - 14	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	Any time at visit	All parameters
16, 18, 20, 21	16, 20, 24, 26	Any time at visit	All parameters
22 - 35	39, 52, 65, 78, 91, 104, 117, 130, 143, 156, 182, 208, 234, 260	Any time at visit	All parameters

Table 12-3, Sampling for Haematology, Coagulation and Urine Parameters

Visit number	Week in relation to IMP administration	Time point at Visit	Parameter to be measured
1	Maximum 6 weeks prior to anticipated IMP administration	Any time at visit	All parameters [a)]
2	-	Prior to IMP administration	All parameters [a)]

6, 10, 14	4, 8, 12	Any time at visit	All parameters [a)]
16, 18, 20, 21	16, 20, 24, 26	Any time at visit	All parameters [a)]
22 - 35	39, 52, 65, 78, 91, 104, 117, 130, 143, 156, 182, 208, 234, 260	Any time at visit	All parameters [a)]

[a)] Lupus anticoagulant and antithrombin will be measured only at Visit 1

12.2.5 Vector DNA

Sampling of the following types of matrix will be performed to determine Vector DNA levels: Blood, saliva, nasal secretions, urine, faeces and semen. Sampling will be initiated at Visit 2 and will continue at the time points given in Table 5-2 (Visits 1 - 21), Table 5-3 (Visit 2, overnight stay), and Table 5-5 (Visits 22 – 35).

Sampling should continue for the individual subject and for a specific matrix until 3 consecutive negative samples have been detected for the subject for that particular type of matrix.

Based on the wish of the subject, faeces and semen samples can be collected at home prior to attending the visit (at the visit day or at the day before the visit day).

12.2.6 Neutralizing Antibodies to AAV5

Blood sampling for measurement of neutralizing antibodies to AAV5 will take place at the following time points:

- at Visit 1 (Screening Visit)
- at Visit 2 (prior to IMP administration)
- at Visits 3 - 6 (weekly in weeks 1 - 4)
- at Visit 21 (week 26)
- at Visit 23 (week 52 / year 1)
- at Visit 27 (week 104 / year 2)
- at Visit 31 (week 156 / year 3)
- at Visit 33 (week 208 / year 4)
- at Visit 35 (week 260 / year 5)

The Investigator will be informed of the value at Screening for the purpose of evaluation of exclusion criterion no. 3.

12.2.7 Total Antibodies to AAV5

Blood sampling for measurement of total (IgG and IgM) antibodies to AAV5 will take place at the following time points:

- at Visit 1 (Screening Visit)
- at Visit 2 (prior to IMP administration)
- at Visits 3 - 6 (weekly in weeks 1 - 4)
- at Visit 21 (week 26)

- at Visit 23 (week 52 / year 1)
- at Visit 27 (week 104 / year 2)
- at Visit 31 (week 156 / year 3)
- at Visit 33 (week 208 / year 4)
- at Visit 35 (week 260 / year 5)

12.2.8 AAV5 capsid-specific T cells

Sampling for the measurement of AAV5 capsid-specific T cells will take place at the following time points:

- at Visit 2 (prior to IMP administration)
- at Visits 3 - 14 (weekly in weeks 1 - 12)
- at Visits 15 - 21 (bi-weekly in weeks 14 - 26)

12.2.9 Anti-FIX Antibodies

Blood sampling for measurement of anti-FIX antibodies will take place at the following time points:

- at Visit 2 (prior to IMP administration)
- at Visit 21 (week 26)
- at Visit 23 (week 52 / year 1)
- at Visit 27 (week 104 / year 2)
- at Visit 31 (week 156 / year 3)
- at Visit 33 (week 208 / year 4)
- at Visit 35 (week 260 / year 5)

12.2.10 FIX Inhibitors

Blood sampling for measurement of FIX inhibitors (Nijmegen modified Bethesda assay at a central laboratory) will take place at the following time points:

- at Visit 1 (Screening Visit)
- at Visit 2 (prior to IMP administration)
- at Visit 14 (week 12)
- at Visit 21 (week 26)
- at Visit 23 (week 52 / year 1)
- at Visit 27 (week 104 / year 2)
- at Visit 31 (week 156 / year 3)
- at Visit 33 (week 208 / year 4)
- at Visit 35 (week 260 / year 5)

12.2.11 FIX Recovery

Measurement of FIX recovery (C_{max}) and incremental recovery measured as increase in activity per unit infused (IU/ml per U/kg) at 30 min after infusion of a dose of FIX will be performed at Visit 2. Additionally, measurement of FIX recovery and incremental recovery should be done at suspicion of FIX inhibitor (see also Section 12.3.10) or at increase in bleeding frequency, as judged by the Investigator.

At each occasion, a FIX challenge dose of 40 U/kg should be administered while at the clinical trial site. A blood sample should be drawn just prior to FIX dosing and at 30 minutes after FIX dosing. The blood sample should preferably be drawn from a vein different from the vein used for FIX infusion.

Date of sampling, times of blood sampling (pre and post FIX administration) and time of FIX administration will be recorded.

FIX activity will be measured at a central laboratory using the one-stage aPTT assay and the chromogenic/amidolytic assay.

12.2.12 Inflammatory Markers

Blood sampling for measurement of IL-1β, IL-2, IL-6, INFγ and MCP-1 will take place prior to IMP administration and in the period up to 18 weeks after IMP administration:

- at Visit 2 (prior to IMP administration)
- at Visits 3 - 14 (weekly in weeks 1 - 12)
- at Visits 15 - 17 (bi-weekly in weeks 14, 16, 18)

12.3 Other Assessments

12.3.1 Local Laboratory Sampling for Management of Subjects and Eligibility Check

Table 12-4 provides the laboratory parameters to be measured by the local laboratory, and specifies which samples should be drawn at each visit.

Table 12-4, Local Laboratory Sampling

Visit number	Week in relation to IMP administration	Time point at Visit	Parameter to be measured
1	Maximum 6 weeks prior to anticipated IMP administration	Any time at visit[1)]	FIX activity[2)] FIX inhibitors[3)] Liver enzymes (AST/ALT)
2	-	Prior to IMP administration	FIX activity[2)] FIX inhibitors[3)] Liver enzymes (AST/ALT)
2	-	24 hours after IMP administration	Liver enzymes (AST/ALT)
3 - 14	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	Any time at visit[1)]	FIX activity[2)] FIX inhibitors[3)] (v14 only) Liver enzymes (AST/ALT)
3b – 14b	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	Any time at visit[1)]	FIX activity[2)] Liver enzymes (AST/ALT)
15 - 20	14, 16, 18, 20, 22, 24	Any time at visit[1)]	FIX activity[2)] Liver enzymes (AST/ALT)
21	26	Any time at visit[1)]	FIX activity[2)] FIX inhibitors[3)] Liver enzymes (AST/ALT)
22 - 35	39, 52, 65, 78, 91, 104, 117, 130, 143, 156, 182, 208, 234, 260	Any time at visit[1)]	FIX activity[2)] FIX inhibitors[3)] (v23, 27, 31, 33, 35 only)

1) For subjects on prophylactic FIX replacement therapy, attempts should be made to allow blood sampling for FIX activity at time points where FIX activity is expected to be at lowest justifiable levels (see Section 12.1.1).
2) The analysis method applied at the local laboratory for FIX activity should be one of the following:
   - One-stage aPTT assay
   - Amidolytic/chromogenic assay

   Preferably, the same type of assay is applied consistently for the individual subject throughout the entire trial period.
3) The analysis method applied at the local laboratory for FIX inhibitors should be one of the following:
   - Nijmegen modified Bethesda assay
   - Bethesda assay

   Preferably, the same type of assay is applied consistently for the individual subject throughout the entire trial period.

The Investigator should arrange with the local laboratory that analysis results are provided on the same day, or the day after, blood sampling has taken place.

The Investigator should enter analysis results, as well as related reference ranges and analysis method applied (if applicable) in the eCRF. In addition, the local laboratory result reports should be kept in the subject's medical record.

12.3.2 FIX Protein Concentration

Blood samples for FIX protein concentration (FIX:Ag) will be collected at all visits. At Visit 2, blood sampling will take place prior to IMP administration.

Throughout the entire trial period (from Visit 1 and onwards) where a subject is on prophylactic FIX replacement therapy, it will be the aim to draw blood samples at time points where FIX protein concentration is expected to be at the lowest levels. To the extent visit

windows allow, the Investigator and/or study nurse will collaborate with the subject to schedule visits to take place on days where prophylactic FIX replacement therapy is planned to be administered. At these visits, blood sampling will then take place just prior to administration of prophylactic FIX replacement therapy, which will then be administered at the clinic instead of at home.

FIX protein concentration in plasma will be measured at a central laboratory.

12.3.3 Demographics and Disease Characteristics

At Visit 1 (Screening Visit), date of birth, race, ethnic group and gender will be recorded according to local regulations. In addition, medical history and concomitant illness(es), (disease related) surgical history, haemophilia B history, and history and status of FIX medication and bleeding will be recorded.

12.3.4 FIX Gene Mutation

Available information on FIX gene mutation will be collected at Visit 1 (Screening Visit) and recorded.

If information on FIX gene mutation is not available, a blood sample for the purpose of FIX gene sequencing analysis will be collected (preferably at Visit 1, but otherwise at a later time point during the subject's trial participation), but only if separate informed consent is given by the subject.

Subjects who do not wish to participate in the FIX gene sequencing analysis may still participate in the trial and will not be required to withdraw from the trial if they withdraw consent for the FIX gene sequencing analysis.

Gene sequencing analysis will be performed at a central laboratory.

12.3.5 Medical History and Concomitant Illnesses

Medical history is any previous medical condition or surgical event, i.e. a condition/event that started prior to Visit 1 (Screening Visit), but is not ongoing at Visit 1. A concomitant illness is a medical condition that is ongoing at Visit 1. Information on bleeding history and haemophilia B disease related surgical events and joint status will be reported separately (see Sections 12.3.6 and 12.3.7, respectively).

At Visit 1, information on relevant medical history will be obtained and recorded. The following conditions and events will be considered relevant (bleeding events excluded):

- Any surgical event or any chronic or ongoing medical condition, regardless if it requires/required therapy or not
- Any medical condition or surgical event that has resulted in sequelae
- Any isolated or one-off medical condition or surgical event that has occurred within 1 year prior to Visit 1 irrespective of the outcome of the event.
- Any isolated or one-off medical condition or surgical event that has resolved without sequelae and occurred more than 1 year prior to Visit 1 if judged relevant by the

Investigator (for example conditions that the Investigator evaluates could re-emerge over time, e.g. cancers).

Furthermore, at Visit 1 information on any medical condition ongoing at Visit 1 will be obtained and recorded.

12.3.6 History and Status of Bleeding

At Visit 1 (Screening Visit) information on the following bleeding history data will be collected:

For all subjects:

- Number of exposure days prior to trial entry. An exposure day is a day where the subject received one or more infusion(s) of FIX replacement therapy. Additional information will be recorded for FIX replacement therapy administered during the last 7 days prior to trial entry (trade name of FIX medication, date & time of administration, units per administration).

For subjects receiving prophylactic FIX replacement therapy:

- Number of months on prophylactic FIX replacement therapy for the last 12 months
- Dose and frequency of dosing; in case the subject is on intermittent prophylactic FIX replacement therapy, each regimen followed in the last 12 months should be reported
- Recombinant or plasma FIX product
- Number of treatment requiring bleeding episodes in the last 12 months during each specific prophylactic treatment regimen
- Average number of units to treat a bleed during each specific prophylactic treatment regimen during the last 12 months
- Number of bleeding episodes prior to initiation of prophylactic FIX replacement therapy.

For subjects receiving on-demand FIX replacement therapy only:

- Number of months on on-demand FIX replacement therapy for last 12 months
- Number of treatment requiring bleeding episodes in the last 12 months during each specific treatment regimen
- Recombinant or plasma FIX product
- Average number of units to treat a bleed during each specific on-demand FIX replacement therapy regimen during the last 12 months.

12.3.7 Information on Haemophilia B Disease Related Surgeries and Joint Status

Information on history of haemophilia B disease related surgery will for all subjects be collected at Visit 1.

Information on joint status, target joints[4] and range of motion will for all subjects be recorded at Visit 1 as well as every 12 months after IMP administration, i.e. at Visit 23, 27, 31, 33 and 35.

[4] Definition of target joint: A joint which the subject has bleed into in the last 6 months at least 3 times

Data include:

- Haemophilia B disease related surgery
  - Date of surgery
  - Surgical event
  - Preventive treatment during surgery: Recombinant or plasma product
- Joint status (Haemophilia Joint Health Score version 2.1). The instruction manual for this assessment will be contained in the Trial Procedures Manual and must be followed. Preferably, the same assessor will consistently perform the assessment on the individual subject throughout the entire trial period
- Number and location of target joints
- Range of motion of joints.

12.3.8 Body Mass Index

At Visit 1 (Screening Visit), height (without shoes) will be measured and recorded, rounded to the nearest centimetre.

At Visits 1, 2 and 21-35, body weight (without overcoat and shoes) will be measured and recorded, rounded to the nearest kilogram. Body mass index will be calculated automatically, based on eCRF entries of height and body weight.

12.3.9 Laboratory Parameters for Evaluation of Subject Eligibility

At Visit 1 (Screening Visit) blood samples will be drawn for the purpose of measurement of the following laboratory parameters: HIV, $CD4^+$, HIV viral load, HBsAg, HBeAg, HBV DNA and HCV RNA.

As described in Section 12.3.1, a blood sample will be drawn at Visit 1 (Screening Visit) for the purpose of measurement of FIX inhibitor by the local laboratory.

As described in Section 12.2.6 at Visit 1 (Screening Visit) a blood sample will be drawn for the purpose of measurement of neutralizing antibodies to AAV5.

Furthermore, as described in Section 12.2.4, Visit 1 blood and urine samples will be drawn for analysis of serum chemistry, haematology, coagulation and urine parameters.

For the purpose of evaluation of Exclusion Criterion 2, the Investigator will use the Visit 1 (Screening) laboratory result from the local laboratory. The Investigator will be informed by the central laboratory about the Screening laboratory results for the purpose of evaluation of Exclusion Criteria 3, 4, 5, 6, 8 and 9.

If pre-determination of subject eligibility is desired by the Investigator in advance of the conduct of the full screening visit (Visit 1), an additional visit can be performed during which a selection of laboratory parameters (neutralizing antibodies, HIV, $CD4^+$, HIV viral load, HBsAg, HBeAg, HBV DNA and HCV RNA) can be measured (see Table 5-2, additional visits). When pre-determination of subject eligibility is established and the subject continues

into the trial, a full screening visit (Visit 1) needs to be conducted (see Table 5-1 and Table 5-2, Visit 1), however if the timing of the additional visit is within 2 weeks of Visit 1 there is no need to repeat the laboratory parameters previously measured.

12.3.10 Further Testing of Subjects with a Suspected FIX Inhibitor

A subject is said to suffer from FIX inhibitors if tested positive for FIX inhibitors at 2 consecutive tests (as measured by the local laboratory), performed preferably within 2 weeks.

If a subject has confirmed FIX inhibitor and continues with no change to treatment type for 6 weeks and the FIX inhibitor test is negative after that time, the FIX inhibitor is classified as transient.

12.3.11 Laboratory Samples for Future Research

Additional blood samples for the purpose of potential future research in the haemophilia B disease area (including development and validation of assays to support efficacy assessments) will be drawn at screening (Visit 1), baseline (i.e. pre-IMP time point during Visit 2) and at the following time points after IMP administration: 5 weeks, 10 weeks, 26 weeks and 52 weeks (Visit 7, 12, 21 and 23).

These additional blood samples will only be drawn if separate informed consent is given by the subject. Subjects who do not wish to donate blood samples for the purpose of potential future research may still participate in the trial and will not be required to withdraw from the trial if they withdraw consent for the potential future research.

The procedures for the collection, processing, storage and shipment of these blood samples are described in the Laboratory Manual.

12.4 General Information Regarding Laboratory Sampling and Results

All laboratory assessments will be conducted at a central laboratory, except FIX activity assay for monitoring of subjects (Section 12.3.1), FIX inhibitor assay for monitoring of subjects and for eligibility check (Section 12.3.1) and liver enzymes (AST and ALT) (Section 12.3.1) which will be conducted locally.

Dates and times of sampling will be recorded.

Detailed procedures for the collection, processing, storage and shipment of laboratory samples are described in the Laboratory Manual. This manual as well as all material such as test tubes and labels will be provided by the coordinating central laboratory.

After the laboratory samples have been analysed, they will remain stored for potential re-analysis at any time during the trial to a maximum of up to 1 year after the trial has been completed, before being destroyed. Exceptions are the future research blood samples, which will be stored and used for medical research until there is no sample remaining.

The Investigator will be provided with laboratory results at regular intervals for review and sign-off. Any abnormality, judged by the Investigator as a clinically relevant worsening since the first measurement, i.e. at Visit 1 or Visit 2, should be reported as an AE, unless the laboratory abnormality is associated with an already reported AE.

Any report of erroneous results in the following parameters from Visit 21 and onwards should prompt that the subject is called in for an Additional Visit to have blood sample(s) drawn for the purpose of re-measurement:

- FIX activity measured at local laboratory
- FIX activity measured at central laboratory
- FIX inhibitors measured at local laboratory
- FIX inhibitors measured at central laboratory
- Coagulation parameters
- AST, ALT
- FIX recovery and incremental recovery.

The Additional Visit should preferably take place within 1 week after the report of the erroneous result(s).

12.5 Total Blood Volume

Blood samples will be collected for efficacy, safety and screening assessments. For each subject, the total blood volume in the first half year, including screening, is expected to be 1906 mL (excluding any additional visits). For the remaining 4.5 years of the study, the total blood volume is expected to be 684 mL. Therefore, the total blood volume for subjects completing the study will be 2590 mL. The maximum blood volume per additional visit (during tapering phase or any other time) is 130 mL.

13. Safety Reporting

13.1 Adverse Event Definitions

An adverse event (AE), an adverse drug reaction (ADR) and a serious adverse event (SAE) are defined according to ICH Guideline E2A.

An AE is any untoward medical occurrence in a subject administered the IMP and which does not necessarily have a causal relationship with this IMP or the IMP administration procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, see Section 12.4), symptom, or disease temporally associated with the use of the IMP including the IMP administration procedure.

An ADR is an untoward and unintended response to the IMP related to any dose administered. A causal relationship between the IMP and the AE is at least a reasonable possibility.

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (this 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 that hypothetically might have caused death if it was more severe)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is judged medically important by the Investigator (this refers to an event, not resulting in any of the outcomes listed above, but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed)

In the following situations, events are not defined as an AE:

- Medical or surgical procedure (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the trial that do not worsen
- Condition(s) for which pre-planned procedure(s) have been recorded at Visit 1, including hospitalization(s), unless the condition(s) for which the procedure and/or hospitalization was planned has worsened from the first trial related activity after the subject has signed the informed consent form
- Concomitant illness identified during the screening procedures will be recorded as medical history. However, whenever symptoms for these condition(s) worsen and/or become serious, then these events must be reported as an AE or SAE, as applicable.

13.2 Adverse Event Assessment Definitions

13.2.1 Severity

The Investigator should assess the severity of all AEs according to the following definitions:

- Mild: Awareness of symptoms, sign, illness or event that is easily tolerated.

- Moderate: Discomfort sufficient to cause interference with usual activity.
- Severe: Incapacitating with inability to work or undertake further normal activities.

Note the distinction between seriousness and severity: The term severe is used to describe the intensity of the event and a severe event is not necessarily serious (e.g. a severe headache would probably not constitute an SAE; however a mild myocardial infarction could constitute an SAE). The seriousness criteria serve as a guide for defining regulatory reporting obligations.

If an AE changes severity over time, the severity of maximum intensity should be reported.

13.2.2 Relationship to IMP

Assessment of causality is based on the following considerations: associative connections (time and/or place), pharmacological explanations, previous knowledge of the drug, presence of characteristic clinical or pathological phenomena, exclusion of other causes, and/or absence of alternative explanations.
The Investigator will be asked to assess causal relationship to the trial treatment according to the following classifications:

- *Probably related*: An AE with a reasonable time sequence to administration of the IMP, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfil this definition.

- *Possibly related*: An AE with a reasonable time sequence to administration of the IMP, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

- *Unlikely related***:** An AE with a temporal relationship to IMP administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

- *Not applicable*: This assessment can be used e.g. in cases where the subject did not receive any treatment with IMP.

13.2.3 Outcome

The Investigator will be asked to record the most appropriate outcome of the following:

- Recovered
- Recovered with sequelae
- Recovering
- Not recovered
- Fatal
- Unknown

13.3 Reporting of Adverse Events

All events meeting the definition of an AE must be reported in the period starting at the first visit during which any trial related activity takes place until the end of trial participation. At each visit the subject will be asked about AEs in an objective manner, e.g.: "Have you experienced any problems since the last visit?". Only medically qualified personnel (Investigators) must assess AEs.

AEs must be reported in the source data and the eCRF. The diagnosis will be recorded, if available and applicable. If no diagnosis is available each sign and symptom will be recorded as individual AEs.

Recurring AEs should be reported separately, i.e. with separate start date and time and stop date and time.

13.4 Prompt Reporting of SAEs and Other Events to uniQure

SAEs, AEs qualifying for special notification and disease-related bleeding episodes related to the IMP and/or with fatal outcome, must be reported as described in the following table once the Investigator determines that the event meets the protocol definition for that event.

	Initial Reports		Follow-up Information on a Previous Report	
Type of Event	**Time Frame**	**Documents**	**Time Frame**	**Documents**
All SAEs	24 hours	SAE form	72 hours*	SAE form
All AEs qualifying for special notification as defined in Section 13.6	24 hours	SAE form	72 hours*	SAE form
Disease-related bleeding episodes related to the IMP and/or with fatal outcome as defined in Section 13.8	24 hours	SAE form	72 hours*	SAE form

* If however, in the opinion of the Investigator, the follow-up information may have implications for the safety of other subjects, the follow-up information is to be reported immediately (i.e. within 24 hours).

The information will be reported on a paper SAE form and will include assessment of severity, causal relationship to the IMP or trial procedures, action taken, outcome, and a narrative description of the course of the event. Additional information may be subsequently provided.

The paper SAE form and all other relevant documents supporting the reported SAE or AE qualifying for special notification (e.g. diagnostic procedures, hospital records, autopsy reports) must be faxed or scanned/e-mailed to

CCI [redacted]
24 hour Hotline: PPD
F: PPD
E-mail: PPD

The Independent Ethics Committees/Institutional Review Boards (IECs/IRBs) and regulatory authorities will be notified of SAEs according to current regulation and local requirements.

SAEs occurring to a subject after the subject has completed the clinical trial and for which a reasonable possibility of a causal relationship is assessed by the Investigator, should be reported by the Investigator to the sponsor if the Investigator becomes aware of them regardless of the time that has elapsed (post-trial events).

13.5 Regulatory Reporting Requirements for SAEs and Other Events

Prompt notification by the Investigator to uniQure of SAEs, AEs qualifying for special notification and disease-related bleeding episodes related to the IMP and/or with fatal outcome is essential, so that legal obligations and ethical responsibilities towards the safety of subjects are met.

uniQure has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. uniQure will comply with ICH/Food and Drug Administration (FDA)/European Medicines Agency (EMA) and country-specific regulatory requirements relating to safety reporting to the regulatory authority, IECs/IRBs and Investigators. Details are laid down in the safety reporting plan which will comply with these regulatory requirements (e.g. 21CFR 312.32).

Expedited reporting of suspected unexpected serious adverse reactions will be taking place within 7 or 15 days depending on fatal/life threatening status, seriousness, expectedness and causality from all clinical trials globally (suspected unexpected serious adverse reaction [SUSAR] reporting in EU and IND safety reports in the US). Any suspected adverse reaction that is both serious and unexpected according to the Investigator's Brochure will be reported provided there is evidence to suggest a causal relationship between the drug and the AE.

Relevant follow-up information to an SAE IND safety report will be submitted as soon as the information is available.

Information about SUSARs is also forwarded to Investigators and IECs/IRBs as necessary.

An Investigator who receives an SUSAR describing an SAE(s) or other specific safety information (e.g. summary or listing of SAEs) from uniQure will file it with the Investigator's Brochure and will notify the IEC/IRB, if appropriate according to local requirements.

In addition to submission of SAEs, an annual Development Safety Update Report (DSUR) will be prepared and submitted to the EMA, FDA, and locally if applicable according to the development international birth date (DIBD).

13.6 Adverse Events Qualifying for Special Notification

New AEs potentially related to the conduct of the trial or the development of the Advanced Therapy Medicinal Products (ATMP) and likely to affect the safety of the subjects should be

reported and followed in the same manner as SAEs (see Section 13.3 and Section 13.7). Note that the AEs may be serious or non-serious by definition (see Section 13.1).

In addition, the following (S)AEs qualify for special notification as they are seen as safety issues of particular concern for ATMPs (ENTR/F/2/SF/dn D(2009) 35810. Brussels, 03/12/2009) and gene therapy medicinal products (EMEA/CHMP/GTWP/60436/2007):

- AEs related to the IMP administration procedure (intravenous infusion)
- Suspected or confirmed cases of opportunistic or serious infections that in the Investigator's opinion might be related to the IMP
- Unexpected reactions (e.g. hypersensitivity, immunological, toxic or other as consequence of a change in the construction or function of the viral vector [e.g. generation of replication competent virus])
- AEs related to product failure (including lack of efficacy)
- Confirmed FIX inhibitors
- Thromboembolic events
- Transaminase elevation > 1.5 times baseline value
- Allergic reactions
- AEs that could be related to potential off-target expression
- Development of any new/recurrent cancer.

13.7 Follow-up on Adverse Events

All AEs should be followed until they have reached a "final outcome" (recovered, recovered with sequelae, recovering, not recovered, fatal or unknown) or the subject's participation in the trial ends, whichever comes first.

Severe, non-serious AEs assessed as "Probably related" or "Possibly related" to IMP and all SAEs and AEs qualifying for special notification (regardless of their relationship to IMP) still ongoing after ended trial participation, should be followed on a regular basis according to the Investigator's clinical judgment until a "final outcome" has been established.

The outcome "recovering" can be used as the "final outcome" for events that are stabilized (i.e. no further worsening is expected) and expected by the Investigator to resolve over time.

The outcome "not recovered" can be used as the "final outcome" for events that are not expected to resolve over time (e.g. cancer).

If a subject dies during the reporting period attempts should be made to obtain biopsy material to perform assay for replication competent retrovirus or other relevant part of the gene therapy medical product and to ascertain the cause of the death (EMEA/CHMP/GTWP/60436/2007).

13.8 Reporting Requirements for Disease-Related Bleeding Episodes

Disease-related bleeding episodes evaluated by the Investigator as part of the underlying disease should not be reported as AEs unless related to the IMP (including bleeding episodes due to lack of efficacy of the IMP) or if the bleeding has worsened as compared to the subject's normal bleeding pattern, as evaluated by the Investigator. In case a bleeding episode

is meeting one or more of the SAE criteria or is related to major surgery requiring in-patient hospitalization, it must be reported as an SAE.

13.9 Pregnancies

Pregnancies of the subject's sexual partner will be recorded separately from AEs, but will be reported in a manner identical to the reporting of SAEs, however via the use of a pregnancy reporting form instead of a SAE form.

All attempts will be made to record all pregnancies occurring during this trial for which the subject received IMP and where the subject's sexual partner has become pregnant after IMP administration.

All attempts will be made to follow-up the pregnancy until the outcome of the pregnancy has been determined and to capture information on the development of the infant in the period up until and including the age of 1 year. This information will only be collected if separate informed consent is given by the subject and the subject's sexual partner.

Any report of a congenital abnormality/birth defect is an SAE and should be reported as such. Any complication of a pregnancy occurring during this trial, including elective termination for medical reasons, must be reported with the pregnancy reporting form.

13.10 Occupational Exposure

Occupational exposure refers to the exposure to a medicinal product as a result of one's professional or non-professional occupation and will be reported in a manner identical to the reporting of SAEs, however via the use of an occupational exposure form instead of a SAE form.

Occupational exposure with or without an AE, should be reported within 24 hours of becoming aware of the exposure.

14. Statistical Methods

A Statistical Analysis Plan will be prepared prior to the first interim analysis. Overall principles are described below.

14.1 Sample Size Calculation

No formal sample size calculation was made. The choice of 5 to 7 subjects in each cohort is considered sufficient to capture commonly occurring AEs (Percy et al., 2010).

14.2 Statistical Methods

All reporting will be made for the Full Analysis Set. Reporting may be made for other analysis populations, or subgroups of subjects based on for example genotype.

Data will be presented in tables and listings following the principles in ICH E3 guideline regarding structure and content of clinical reports. Data will also be presented graphically by subject and for selected endpoints all data will be presented.

Summary tables will show number of values and when applicable number of missing values.

Categorical data will be presented with number of cases and percentage of total number of cases. Continuous data will be presented with mean and SD, median, minimum and maximum as appropriate.

Continuous data will be shown in listings with the same precision as captured.

Subject level summaries will be presented adjusted for observation time. This includes but is not limited to annualized rate of selected signs and symptoms, annualized bleeding rate, average value for laboratory parameter or area under the time-profile curve normalized with time.

Laboratory parameters will be presented by visit and include categorization of parameters into low/normal/high where applicable.

Prophylactic and on-demand FIX replacement therapy will be summarized according to type of medication. Other concomitant treatment will be presented by therapeutic grouping presenting number of subjects and number of treatments.

No formal statistical hypothesis will be tested. All confidence intervals represent exploratory analyses and will not be related to formal statistical hypothesis tests. No correction for multiplicity will be applied.

14.2.1 Primary Efficacy Endpoint Analysis

AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class and preferred term presenting the number of subjects and number of events. Furthermore, summary tables will be made presenting severity for each event, and using the maximal severity for event. Separate summaries will be made for selected AEs.

14.2.2 Secondary Efficacy Endpoint Analysis

FIX Levels in Plasma
Data will be presented by subject, time point and dose level as summary tables and graphically.

Adjustment for time since most recent administration of FIX replacement therapy may be made, for example reporting by time less than 72 hours versus more than 72 hours after (reflecting prophylaxis free assessment). If applicable, the presentation will include summary statistics and confidence intervals for difference in FIX concentration between time-periods.

If applicable, the FIX level will be analysed using Non-Linear Mixed Models, possibly after transforming the data, and may include subject level random effect or subject level covariate to describe dependency between measurements. The analysis may include information on dose and timing of FIX replacement therapy. If relevant, the analysis may be extended to include modelling of values below lower level of quantification. The modelling may include subject level information on type of FIX replacement therapy, half-life, and if available previous knowledge of subject specific half-life for the FIX replacement therapy.

Number, Severity and Type of Bleeding Episodes
Bleeding episodes will be summarized by subject and overall, and will include number, duration and severity. Separate summaries may be made for bleeds depending on type and location, including target joints, and also summaries excluding recurrent bleeds.

Annualized bleeding rates overall and by severity will be presented. If relevant, summaries will be made using timing representing subject specific periods for pre-tapering, tapering period, and post tapering period until 26 weeks, and post 26 weeks.

Furthermore, the number of bleedings may be analysed using models for count data adjusting for time at risk using generalized linear model with Poisson or Negative Binomial distribution. Where description of timing and inter-event duration is of interest, models for time-to-event analysis may be used, such as Cox Proportional models or other parametric model, possibly including subject level frailty parameter. The analysis may include information on dose and timing of FIX replacement treatment, and measured FIX concentration.

Prophylactic and On-demand FIX Replacement Therapy
The use of prophylactic and on-demand FIX replacement therapy in general will be summarized by subject and overall. If relevant, summaries will be made using timing representing subject specific periods for pre-tapering, tapering period, and post tapering period until 26 weeks, and post 26 weeks.

14.2.3 Patient Reported Outcome Data

For CCI [redacted], the scale scores will be listed by subject and visit and summarized as appropriate.

14.3 Subject Population(s) for Analysis

The Full Analysis Set will consist of all dosed subjects and will be used for reporting. If applicable, further analysis sets will be defined, such as a Per Protocol Analysis set.

14.4 Significance Level

No formal statistical testing will be performed. Unless otherwise noted statistical tests will be reported using a significance level of 0.05. Confidence intervals will be presented as 95% 2-sided confidence intervals.

14.5 Termination Criteria

Not applicable, as no formal procedure based on statistical decision rules is defined.

14.6 Handling of Missing Data

All data will be listed, including unused and spurious data. Imputation of missing or partial data will not be done unless explicitly described. In case of imputation of missing data, both the original missing or partial data and the imputed values will be listed.

14.6.1 Handling of Missing Data for Bleeding Episodes

Bleeding episodes will be recorded with start and end date and time. A bleeding episode is classified as a re-bleed, if there is less than 72 hours between start and stop of previous episode (definition of re-bleed in Section 12.1.2). In case of partial start and stop date, the classification will be made using a conservative approach.

14.7 Deviation Reporting

Deviation(s) from the planned statistical analysis plan will be described and justified in the protocol and/or in the final CSR.

14.8 Interim Analysis

Interim analyses will be performed at the request of the data monitoring committee and at the request of the sponsor to enable reporting of adequate data at selected time points during the course of the trial. Descriptive statistics alone will be used for the interim analyses.

Formal interim analyses will occur after all subjects have been followed for at least 12 weeks (i.e. completed Visit 14) and at least 52 weeks (i.e. completed Visit 23). The data from the 52-week analyses will be reported in an interim CSR.

15. Data Monitoring Committee

A data monitoring committee will be established to:

- perform on-going safety surveillance throughout the entire trial period
- evaluate available safety data from the first subject in each cohort and provide a recommendation to the sponsor regarding dosing of the second subject
- evaluate available safety data from the second subject in each cohort and provide a recommendation to the sponsor regarding dosing of the subsequent subjects in a cohort
- evaluate available safety data from subjects in Cohort 1 and provide a recommendation to the sponsor regarding dosing of subjects in Cohort 2

The data monitoring committee's review of available data prior to dosing of subjects within each cohort as well as between cohorts may reveal safety issues requiring further investigation prior to dosing of the next subject(s). The data monitoring committee will align their recommendation(s) with the Trial Stopping Rules as given in Section 19.2.

The data monitoring committee will consist of at least 2 external and independent experts, of which one will be the chairman. In addition, at least one of the external independent experts should be medically qualified.

Details on the composition, tasks and responsibilities of the data monitoring committee will be described in the data monitoring committee charter.

16. Ethics

16.1 Independent Ethics Committee / Institutional Review Board (IEC/IRB)

Before implementing this trial, the protocol, the proposed information sheet and informed consent form, the subject diaries and other documents as required, will be reviewed by a properly constituted IEC / IRB.

A signed and dated statement stating that the documents have been approved by the IEC / IRB and regulatory authority will be obtained before initiation of the trial.

The name of the IEC / IRB chairman and the members of the IEC / IRB will be collected as well as a statement that the IEC / IRB works in accordance with the principles of GCP. The IEC / IRB will receive updates on the trial progress according to local regulations.

16.2 Subject Information and Informed Consent

Prior to or no later than at start of the screening visit, i.e. before any trial related activity takes place, the Investigator or a qualified designee will explain to the potential subject the aims, methods, reasonably expected benefits and potential hazards of the trial and any discomfort participation in the trial may entail. Subjects will be informed that participation in the trial is voluntary and that the subject may withdraw from the trial at any time and for any reason. Subjects will be informed that if they choose not to participate, this will not affect the care the subject will receive for the treatment of his or her disease. Finally, subjects will be informed that their records may be accessed by regulatory authorities and authorized sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable laws or regulations.

The informed consent will also contain any additional required information with regards to the nature of the IMP, i.e. being an ATMP (ENTR/F/2/SF/dn D(2009) 35810. Brussels, 03/12/2009).

All explanations will be in layman's language. The subject will be given sufficient time to consider the trial before deciding whether to participate or not. All subjects will be given opportunity to ask questions and will be given sufficient time to consider before consenting. The subjects may choose to be accompanied, e.g. by a family member, during the information process.

After having consented, a copy of the information sheet and the signed informed consent form will be given to the subject.

16.3 Subject Treatment Cards

All subjects will receive a subject treatment card, which has been approved by the sponsor and the IEC / IRB, containing at a minimum:

- The name of the subject
- The Investigator's contact number
- Information regarding the IMP received

17. Quality Control and Quality Assurance

17.1 Compliance with Good Clinical Practice

This protocol is written to be in compliance with the guideline produced by the ICH on the topic GCP (ICH Guideline E6 (R1)), and with the related detailed guidelines on GCP specific to advanced therapy medicinal products, as well as with applicable regulatory requirements in the countries where the trial will take place.

The overall responsible Investigator at each clinical trial site (i.e. the principal Investigator) will agree to conduct the trial in adherence to the instructions and procedures described in the protocol, to the Declaration of Helsinki, to the principles of GCP and to applicable regulatory requirements by signing the Principal Investigator's Agreement page of this protocol.

17.2 Monitoring, Audit and Inspection

Monitoring visits to the clinical trial site will be made periodically in person or virtually during the trial to ensure adherence to the protocol, to the principles of GCP and to applicable regulatory requirements.

The Investigator will ensure that the trial monitor is given direct access to source data (in paper and/or electronic format), enabling the trial monitor to perform data validation.

The clinical trial site may be audited by the sponsor or inspected by regulatory agencies. The Investigator will make sure that he/she and his/her relevant clinical trial site personnel make themselves available during monitoring visits, audits and inspections.

17.3 Source Data Verification

Source data must be available to document the existence of the subject and to substantiate integrity of study data collected. Source data must include the original information related to the study (e.g. electrocardiogram traces, laboratory prints), to the medical treatment, and history of subject. Source data are contained in source documents (original records or certified copies). E-diary entries, CCI entries and medical/hospital records on bleeding episodes will be considered source data.

All data entered in the eCRF should be verifiable in source documents in the subject's medical record, work sheets, or in other records at the clinical trial site.
For each clinical trial site, the location of the individual type of source data will be defined and documented.

The trial monitor will check the eCRF for accuracy and completeness and perform Source Data Verification (SDV). The trial monitor will document SDV of all reviewed sections of the eCRF.

18. Data Handling and Record Retention

18.1 Data Handling

Data from clinical trial sites will be entered in an eCRF.

The Investigator will sign relevant eCRF sections. Only an Investigator (i.e. medically qualified site personnel) can sign data on medical assessments. Any corrections made by the Investigator or authorized staff to the eCRF after original entry will be recorded in an audit trail. The person making the change and the date, time and reason for the change will be identified in the audit trail. Changes to data already approved/signed by an Investigator will require re-signature by the Investigator. The Investigator (principal Investigator or co-Investigator) will sign all subject data in the eCRF by an electronic signature.

18.2 Data from Clinical Trial Sites

In the trial an eCRF system will be used for data capture. The system is fully validated and access at all levels to the system is granted/revoked following vendor procedures, in accordance with regulatory requirements and system requirements (e.g. FDA 21CFR11).

Trial data has to be entered into the eCRF system in a timely manner and no later than 5 working days after the subject visit has taken place.

CCI data will be collected on paper, and subsequent data entry into the eCRF system will be performed by local clinical trial personnel.

Subject reported diary data will be collected in an electronic diary system, with the capability to upload this data to the eCRF system, to facilitate Investigator assessment.

Local laboratory data of FIX activity, inhibitors and liver enzymes will be entered into the eCRF system by clinical trial personnel. Other laboratory data will be added to the trial database directly, prior to database lock.

To aid in CSR reporting of missed visits due to COVID-19, the eCRFs will capture if a visit is missed and reason(s) why.

After the trial database is declared clean and is released to the statistician, a final copy of the database, including metadata, closed queries and the audit trail report, will be stored at the sponsor. The Investigator will receive a copy of the clinical trial site's final and locked data, including metadata, closed queries and the audit trail report, as write-protected pdf-files, directly from the database vendor, before their access to the eCRF system is revoked.

18.3 Coding of Data

Medical history, concomitant illnesses, signs and symptoms will be coded using MedDRA.

Prophylactic and on-demand FIX replacement therapy and other concomitant treatment will be coded using WHO Drug Dictionary (WHO-DD).

18.4 Subject Confidentiality

Subject data will be collected anonymously and the subjects will be identified only by subject ID number consisting of a 2-digit number for the clinical trial site followed by a consecutive 2-digit number for the subject.

18.5 Record Keeping

The trial monitor will instruct the Investigator to maintain source documents and the signed informed consent form(s) for each subject. Furthermore, the trial monitor will instruct the Investigator to maintain essential documents during the trial and to archive essential documents for the duration defined in the ICH Guideline E6, or for 15 years, whichever comes first.

The duration of archiving defined in the ICH Guideline E6 is as follows: Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirements or by an agreement with the sponsor. The sponsor will notify the Investigator when retention of the trial-related records is no longer required.

19. Changes to Trial Conduct

19.1 Protocol Amendments

Prospective protocol waivers are prohibited. Any changes to the protocol will be described in a protocol amendment. Any protocol amendment will be agreed upon (signed) by the principal Investigator and the sponsor prior to its implementation. In addition to Investigator and sponsor signature, substantial amendments will be submitted to the IECs / IRBs and regulatory authorities for approval, prior to their implementation.

The Investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IEC / IRB approval/favourable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, a protocol amendment(s) should be submitted to the IEC / IRB for review and approval/favourable opinion and, if required, to the regulatory authority(ies).

The sponsor will decide if an amendment is to be considered substantial or non-substantial, in adherence with "Detailed guidance for the request for authorization of a clinical trial on a medicinal product for human use to the competent authorities, notification of substantial amendments and declaration of the end of the trial" (CT1, 2010/C 82/01, issued by the European Commission, 2010).

19.2 Premature Termination of the Trial or Dosing Temporarily on Hold

The data monitoring committee will perform review of available safety data prior to dosing of subjects within each cohort as well as between cohorts (see Section 15). This review may reveal safety issues requiring further investigation prior to dosing of the next subject(s) or safety issues requiring the trial being terminated prematurely (see Trial Stopping Rules in Section 19.2.1).

The trial may be terminated prematurely by the sponsor. The reasons may be:

- Safety alert or safety issue that prevents further dosing (see Trial Stopping Rules in Section 19.2.1)
- Recruitment not feasible
- A decision to discontinue development of the IMP.

If the trial is terminated prematurely, the sponsor will notify the IECs/IRBs and regulatory agencies immediately (in writing), including the reason for early termination.

A clinical trial site may be terminated prematurely by the sponsor. The reasons may be:

- Insufficient compliance with trial procedures
- Failure to recruit subjects at an acceptable rate.

19.2.1 Trial Stopping Rules

As the trial is a one-dose trial, stopping rules are relevant only for the recruitment period. Independently of any changes to the recruitment, subjects who have already been dosed will be followed as per protocol to the end of trial.

The data monitoring committee will prior to dosing review data as detailed below and recommend whether:

- The next subject could be dosed
- Further data would be required before the next dosing
- No further dosing should take place i.e. subject recruitment is discontinued.

The following events may lead to a decision to pause dosing, i.e. stop subject recruitment to allow for evaluation and assessment of the implications of the event for further subject dosing:

1. A more than 5-fold increase in ALT, AST or both in one or more subjects after IMP administration which is not manageable by steroid rescue treatment implemented according to American Association for the Study of Liver Diseases (AASLD) guidelines. (Manns et al. 2010)
2. An SAE judged as probably or possibly related to the IMP and which pose either an immediate risks to subject's health or is likely to adversely affect the subject's health long term. This includes events classified as AEs qualifying for special notification (Brussels, 03/12/2009,ENTR/F/2/SF/dn D(2009) 35810; EMEA/CHMP/GTWP/60436/2007) as listed in Section 13.6, if these are judged as probably or possibly related to the IMP. The development of an inhibitor towards FIX in one subject is not a cause for stop of subject recruitment, however the development of an FIX inhibitor in more than one subject should lead to pausing of dosing and further investigations of causal relationship
3. Death of a subject, after having received the IMP, that is judged as related to the IMP
4. The occurrence of a malignancy at any point after gene transfer that is judged as probably or possibly related to the IMP.

Decision to start Cohort 2

If none of the events listed above have occurred the data monitoring committee may recommend that Cohort 2 can be initiated. If one or more of these events have occurred, dosing will be paused while the event(s) is/are further investigated including the implications for further dosing and dose level.

Decision to dose the next subject within a cohort

In addition to the above criteria (and as detailed in Section 10.1), the data monitoring committee will evaluate the available safety data collected during a period of minimum 24 hours after IMP administration to the 1st subject and recommend if and when dosing of the 2nd subject can be initiated. Similarly, after the 2nd subject has been dosed in a cohort, the data monitoring committee will evaluate available safety data collected during a period of minimum 24 hours after IMP administration to this second subject and recommend if and when dosing of the subsequent subjects can be initiated. The dosing of the 3rd, 4th and 5th and possibly 6th and 7th subject within a cohort will be separated by a minimum of 24 hours (see Section 10.1) to allow evaluation of any acute, unexpected AEs considered related to the IMP as well as any clinically relevant changes in CRP.

20. Reporting of Results

20.1 Integrated Clinical Study Report

An interim analysis will be performed after all enrolled subjects have completed at least 52 weeks of follow-up after IMP administration (i.e. completed Visit 23). A database freeze will be performed. Collected efficacy and safety data will be analysed and reported in an interim CSR. The data will be considered locked at that point and will not be changed.

After all subjects have completed the 5-year visit (i.e. Visit 35), all collected safety and efficacy data will be locked, analysed, and reported in a full (5 year) CSR.

The full (5 year) CSR will also summarize contingency measures implemented to manage study conduct due to COVID-19 control measures, protocol deviations due to COVID-19, and the impact COVID-19 had on visit schedules, missed visits, and missing information.

The sponsor will notify the accredited IEC/IRBs and the regulatory authority of the end of the trial within a period of 90 days. The end of the trial is defined as the last subject's last visit.

In case the trial is ended prematurely, the sponsor will notify the IEC/IRB and the regulatory authority within 15 days, including the reasons for the premature termination. Within one year after the end of the trial, the Investigator/sponsor will submit the interim CSR and full (5 year) CSR with the results of the trial, including any publications/abstracts of the trial, to the accredited IEC/IRB and the regulatory authority.

20.2 Public Disclosure and Publication Policy

Basic information of this trial will be posted by the sponsor on the website www.clinicaltrials.gov before the first subject enters the trial.

The sponsor is committed to secure that the results of the trial will be published and presented at (scientific) symposia and/or congresses, whether positive or negative and the Investigators will have free access to review and publish the data generated from the trial. The criteria for authorship as set out by the Committee of Medical Journal Editors (www.icmje.org) will be applied.

The contributorship model will be applied and contributors who do not meet the criteria for authorship will be listed in an acknowledgments section with descriptions of the role of each contributor in order to ensure indexation in the National Library of Medicine.

Publications are subject to the following conditions:

- All information concerning the product and the sponsor's operations (such as patent applications, formulae, manufacturing processes, basic scientific data or formulation information supplied to the Investigator by the sponsor and not previously published) is considered confidential by the sponsor and cannot be disclosed by the Investigator to any third party without the sponsor's prior written approval. The Investigator agrees to use this information only in accomplishing the trial and will not use it for other purposes without the written approval of the sponsor.

- It is understood by the Investigator that the sponsor will use the information developed in this trial in connection with the development of the compound and therefore, may disclose it as required to other clinical Investigators, the licensing authority, or to regulatory agencies of other governments. In order to allow for the use of the information derived from this trial, the Investigator understands that he/she has an obligation to provide and disclose test results and all data developed during this trial to the sponsor.
- Any data from the trial are the property of the sponsor and cannot be published without prior authorization from the sponsor.
- Publications must be drafted with protection of individual privacy, intellectual property and contract rights in mind, and also conform to legislation and current national practices in patent and other laws.
- Publications may not disclose any sponsor confidential information or property without prior written consent. Therefore, any final version of a manuscript based on data derived from this protocol must be submitted to the sponsor prior to submission to the publisher and provide the sponsor with at least 45 days of full review of the manuscript. This is to secure that the sponsor can submit relevant intellectual property information prior to public disclosure of intellectual property right sensitive information.
- The primary publication should be published before, or in parallel with, any secondary publications.

The sponsor will organize the publication process to ensure

- Publication of the results of the trial (primary and secondary publications) in a timely and responsible manner.
- That any publication is written according to appropriate guidelines (Wager et al., 2003; Graf et al., 2009).

21. Financing and Insurance

The financial aspects of the trial will be addressed in a separate agreement between the sponsor and the Investigator/clinical trial site.

The sponsor will cover this trial by means of an adequate insurance of the subject, which will be in place prior to the start of the trial. As evidence of this insurance, a copy of the insurance statement will be provided to the Investigator for storage in the Investigator site file.

22. Administrative Aspects

22.1 Investigators

One principal Investigator and one or more sub-Investigators will be appointed for each clinical trial site. Name and title of the Investigator(s) who is (are) responsible for conducting the trial, and the address and telephone number(s) of the trial site will be contained in other documents such as the Trial Procedures Manual and Clinical Trial Application forms.

One National Coordinating Investigator (NCI) will be appointed for each participating country. The NCI will be responsible for national issues relating to the trial.

22.1.1 International Coordinating Investigator

The International Coordinating Investigator is responsible for approval of the protocol and for approval of the CSR on behalf of all trial Investigators.

22.2 Clinical Trial Sites

Since this is a first in-human trial, the clinical trial sites should be appropriate clinical facilities and the trial should be conducted by trained Investigators who have acquired the necessary expertise and experience in conducting early phase trials and medical staff with appropriate level of training and previous experience of first in-human trials. They should also understand the IMP, its target and mechanism of action.

Clinical trial sites should also have immediate access to equipment and staff for resuscitating and stabilising individuals in an acute emergency (such as cardiac emergencies, anaphylaxis, cytokine release syndrome, convulsions, hypotension), and ready availability of Intensive Care Unit facilities. Procedures should be established between the clinical trial site and its nearby Intensive Care Unit regarding the responsibilities and undertakings of each in the transfer and care of subjects.

22.3 Vendors

The sponsor will engage vendors to perform the following services:

- Laboratory analysis
  - Individual central laboratories will be applied for specific laboratory analyses.
  - One coordinating central laboratory will prepare the Laboratory Manual, sampling kits, perform training of clinical trial site personnel, arrange for courier shipments, manage central preparation and storage of samples.
- Clinical monitoring
- Pharmacovigilance
- Data management
- Statistics
- The provision of the CCI [redacted]
- The provision of the e-diaries

Details on the vendors will be contained in other document(s) such as the Trial Procedures Manual and Clinical Trial Application forms.

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23.1 Publications

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Signature Page

CT-AMT-060-01 - Protocol - protocols

Signed By	Date (GMT)
PPD	PPD
Approved-Internal Approval	

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