CSL Behring

CLINICAL TRIAL PROTOCOL

Trial Title:	Phase IIb, open-label, single-dose, single-arm, multi-center trial to confirm the Factor IX activity level of the serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimized human factor IX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe hemophilia B
Short Title:	Dose confirmation trial of AAV5-hFIXco-Padua.
Protocol Identification:	CT-AMT-061-01
Name of Investigational Product:	AMT-061 (AAV5-hFIXco-Padua; etranacogene dezaparvovec)
IND No.:	CCI
Indication Studied:	Hemophilia B
Developmental Phase of Trial:	IIb
Name of the Sponsor/Company:	CSL Behring LLC 1020 First Avenue King of Prussia Pennsylvania 19406, USA
Name of Monitoring CRO:	Medpace (with oversight by uniQure biopharma B.V.)
Coordinating Investigator:	PPD
Protocol Version and Date:	Version 4.0 (Amendment 3.0) 08 Feb 2022 Version 3.0 (Amendment 2.0) 10 Feb 2021 Version 2.0 (Amendment 1.0) 21 May 2019 Version 1.0 (Original) 30 Nov 2017

This trial, including the archiving, will be conducted in compliance with Good Clinical Practice (GCP) according to the International Council for Harmonisation (ICH) Harmonised Tripartite Guideline (CPMP/ICH/135/95)

CLINICAL TRIAL PROTOCOL SIGNATURE PAGE

Sponsor's Approval

Signature:	Date:
PPD	PPD
PPD, Clinical Development	

INVESTIGATOR'S ACKNOWLEDGEMENT

I have read this

Protocol ID: CT-AMT-061-01

Title: Phase IIb, open-label, single-dose, single-arm, multi-center trial to confirm the Factor IX activity level of the serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimized human factor IX gene (AAV5-hFIXco-Padua, hereafter referred to as AMT-061) administered to adult subjects with severe or moderately severe hemophilia B.

I have fully discussed the objectives of this trial and the contents of this protocol with the Sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the trial, without written authorization from the Sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this trial according to this protocol and any trial specific manuals, to comply with its requirements, to subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with International Council for Harmonisation guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to my participation as an Investigator for this trial to be terminated.

I understand that the Sponsor may decide to suspend or prematurely terminate the trial at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial, I will communicate my intention immediately in writing to the Sponsor.

Investigator Name, Address, and Telephone Number: (please handwrite, print or type)	

Signature: _____ Date: _____

SUMMARY OF CHANGES

Current Protocol Amendment		
Summary of Change(s) Since Last Version of Approved Protocol: See Section 13.2		
Amendment Number: 3.0 (Version 4.0)	**Amendment Date:** 08 Feb 2022	**Global/Country/Site-Specific:** Global

List of All Previous Amendments		
Summary of Change(s) for Previous Amendments: Not Applicable		
Amendment Number	**Date of Amendment**	**Global/Country/Site-Specific**
2.0 (Version 3.0)	10 Feb 2021	Global
1.0 (Version 2.0)	21 May 2019	Global
0.0 (Version 1.0)	30 Nov 2017	Global

EMERGENCY CONTACT INFORMATION

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 electronic Case Report Form (eCRF) within 24 hours of becoming aware of the event. In case the eCRF is temporarily unavailable, the back-up paper SAE form should be completed and submitted to CSL Behring within 24 hours of becoming aware of the event.

CSL Global Clinical Safety & Pharmacovigilance:	
Email:	PPD
Fax:	USA: PPD AUS: PPD GER: PPD

For protocol- or medical-related issues during normal business hours as well as outside of normal business hours, the Investigator must contact both the Medpace and uniQure PPD:

Medpace PPD **:**	**uniQure** PPD **:**
PPD PPD PPD PPD	PPD PPD PPD PPD

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

List of Abbreviations

AAV	adeno-associated virus
AAV2	adeno-associated viral vector serotype 2
AAV5	adeno-associated viral vector serotype 5
AAV5-hFIXco	recombinant adeno-associated viral vector serotype 5 containing the wild-type human factor IX gene, codon-optimized for optimal expression in humans, under control of a liver-specific promoter (AMT-060)
AAV5-hFIXco-Padua	recombinant adeno-associated viral vector serotype 5 containing a codon-optimized Padua derivative of human coagulation factor IX cDNA (AMT-061)
AAV8	adeno-associated viral vector serotype 8
ADR	adverse drug reaction
AE	adverse event
AFP	Alpha-fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APOE	Apolipoprotein E
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATMP	advanced therapy medicinal product
BDD-FVIII	B-domain deleted factor VIII
CCI	CCI
cDNA	complementary deoxyribonucleic acid
CHMP	Committee for Medicinal Products for Human Use
C_{max}	Maximum concentration
CMV	Cytomegalovirus
COVID-19	Coronavirus Disease 2019
CRA	clinical research associate
CRO	Contract Research Organization
CRP	c-reactive protein
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EU	European Union
CCI	CCI
FDA	Food and Drug Administration
FVIII	factor VIII
FIX	coagulation factor IX
GFP	green fluorescent protein
γGT	gamma-glutamyl transferase
gc	genome copies

GCP	Good Clinical Practice
GTWP	Gene Therapy Working Party
h	hour
CCI	CCI
hAAT	human α1-antitrypsin
HBeAg	hepatitis B extracellular antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
CCI	CCI
hFIX	human coagulation factor IX
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
CCI	CCI
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (previously International Conference on Harmonisation)
ICF	Informed Consent Form
iCSR	interim Clinical Study Report
IEC	Independent Ethics Committee
IFNγ	interferon gamma
IgG	immunoglobulin G
IgM	immunoglobulin M
IL-1β	interleukin-1beta
IL-2	interleukin-2
IL-6	interleukin-6
IMP	investigational medicinal product
IND	Investigational New Drug
INR	International Normalized Ratio
CCI	CCI
IRB	Institutional Review Board
ISF	Investigator Site File
IU	international unit
IV	intravenous
LTR	Long terminal repeat
MCP-1	monocyte chemotactic protein-1
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Agency (UK)
NAb	Neutralizing antibody
NHP	non-human primate
PBGD	porphobilinogen deaminase
PCR	polymerase chain reaction
CCI	CCI
CCI	CCI
qPCR	quantitative (real-time) polymerase chain reaction
RNA	ribonucleic acid

SAE	serious adverse event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SOC	System Organ Class
SUSAR	suspected unexpected serious adverse reactions
TEAE	treatment emergent adverse event
UK	United Kingdom
ULN	Upper limit of normal
US	United States
VAS	Visual Analogue Scale
w	week
WFH	World Federation of Hemophilia

Definitions of Terms

Baseline	Baseline is defined as Day 0 prior to investigational medicinal product (IMP) administration (pre IMP)
Continuous routine prophylaxis	Continuous routine prophylaxis is defined as the intent of treating with an a priori defined frequency of infusions (e.g., twice weekly, once every two weeks, etc.) as documented in the medical records.

CLINICAL TRIAL PROTOCOL SYNOPSIS

Protocol Number: CT-AMT-061-01	**Investigational Medicinal Product:** AMT-061 (AAV5-hFIXco-Padua; etranacogene dezaparvovec)
Title of the Trial: Phase IIb, open-label, single-dose, single-arm, multi-center trial to confirm the Factor IX activity level of the serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimized human factor IX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe hemophilia B.	
Planned Number of Subjects: Three subjects are planned to be treated.	
Planned Number of Sites and Site Location(s): It is planned to conduct this trial in approximately three sites in the United States (US) and European Union (EU).	
Coordinating Investigator	PPD
Trial Period (Planned): First Subject Screening: Q2 2018 Last Subject Last Visit: Q3 2023	**Clinical Phase:** IIb
Indication/Trial Population: Male subjects with severe or moderately severe hemophilia B.	
Objectives: **Primary:** To confirm that a single dose of 2 x 10^{13} genome copies (gc)/kg AMT-061 will result in factor IX (FIX) activity levels of ≥5% at six weeks after dosing **Secondary:** To assess further efficacy and safety of 2 x 10^{13} gc/kg AMT-061.	
Rationale: Somatic gene therapy for hemophilia B offers the potential for shift of the disease severity from severe to a moderate or mild hemophilic phenotype or complete amelioration through continuous endogenous production of factor IX protein after a single administration of adeno-associated viral vector particles. Even a small rise in constantly circulating factor IX protein can substantially ameliorate the bleeding phenotype. Robust preliminary efficacy and safety results have been obtained with AMT-060 in an ongoing Phase 1/2 trial (CT-AMT-060-01) in 10 subjects with hemophilia B. No screening failures due to high titers of pre-existing neutralizing antibodies against adeno-associated viral vector serotype 5 (AAV5) were encountered. An increased level of factor IX activity was observed after subjects in the low dose cohort and high dose cohort were treated with the AMT-060 at 5 x 10^{12} gc/kg and 2 x 10^{13} gc/kg, respectively. Clinically relevant endogenous factor IX activity (≥2% of normal circulating factor IX) was established in nine out of 10 subjects across both cohorts (five out of five in the high dose cohort). Continuous, regular prophylaxis was discontinued in eight out of nine subjects previously dependent on prophylaxis (four out of four in the high dose cohort). As of writing, factor IX expression levels remain stable and durable for up to 1.5 years without indications of loss of expression. A shift to mild hemophilia B phenotype (>5% of normal circulating factor IX) was achieved in two out of five subjects in the low dose and in four out of five subjects in the high dose cohort. A reduction/elimination in the use of factor IX replacement therapy and a lowering in the risk of bleeding events over time were demonstrated in both cohorts. The single intravenous (IV) infusion of AMT-060 was safe and well-tolerated in both the low- and high-dose cohorts. The most frequent adverse event (AE) related to gene therapy targeting the liver is alanine aminotransferase (ALT) elevation. One subject in the lower dose cohort and two subjects in the higher dose cohort had mild, asymptomatic, transient elevations of ALT. No change in factor IX activity and no T-cell response were seen in the subjects with ALT elevations. No subjects developed inhibitory antibodies against factor IX. The clinical benefit of AMT-060 was achieved with a factor IX protein-to-activity ratio of approximately 1. Modifying AMT-060 with the implementation of the naturally occurring PADUA variant (2 nucleotide change in the wild-type human factor IX - hereafter referred to as AMT-061) is expected to result in a factor IX protein-to-activity ratio of approximately six. Although AMT-061 has yet to be studied in humans this design modification is anticipated to lead to a higher level of circulating factor IX activity at comparable levels of factor IX protein and thereby increase the likelihood of alleviating the need for exogenous therapy, including the need for on-demand	

treatment of traumatic bleeds and selective prevention of a bleed (e.g., because of upcoming physical activity, sports, etc.).

This isolated modification is a prospectively defined product improvement that will not influence other established safety characteristics of AAV5 at 2 x 10^{13} gc/kg such as the level of factor IX protein expressed with 2 x 10^{13}gc/kg AMT-061, immune response against AAV5, the occurrence of manageable ALT elevations or risk of T-cell responses against transduced cells with potential concurrent loss of efficacy.

In addition to the above, the continuous accumulation of clinical data from the use of AAV5 in hemophilia subjects suggests that AAV5 is associated with a very low risk of inducing immune responses that may lead to loss of the newly induced endogenous factor IX protein expression. Furthermore, low titers of pre-existing antibodies against AAV5 (using a highly sensitive assay), which may demonstrate neutralization of AAV5 transduction in in-vitro assays, have no apparent inhibitory effect in human studies at currently administered dose levels. As a consequence, future trials with AMT-061 (including this trial) will allow enrolment of subjects even in the presence of anti-AAV5 antibodies.

The strong efficacy (protein expression) and safety results obtained during the Phase 1/2 trial with AMT-060 demonstrate 2 x 10^{13} gc/kg to be the optimal dose for use in future trials in terms of safety and efficacy. In addition, accumulated clinical and non-clinical data support the implementation of the prospectively defined product enhancement of AMT-061 at 2 x 10^{13} gc/kg for the pivotal trial. The primary aim of this trial is to confirm that a single dose of 2 x 10^{13} gc/kg AMT-061 will result in factor IX activity levels of ≥5%. An objective of the trial is to assess whether observed factor IX activity levels are within an expected range, to determine if 2 x 10^{13} gc/kg AMT-061 is suitable from efficacy point of view for administration in the pivotal Phase 3 trial. In addition, the safety profile of AMT-061 will be demonstrated.

Investigational Medicinal Product, Dose, and Mode of Administration:

The investigational medicinal product (IMP) is identified as AAV5-hFIXco-Padua (AMT-061; etranacogene dezaparvovec). AMT-061 is a recombinant AAV5 containing the Padua variant of a codon-optimized human factor IX complementary deoxyribonucleic acid (cDNA) under the control of a liver-specific promoter. The pharmaceutical form of AMT-061 is a solution for IV infusion.

The single administered dose of AMT-061 is 2 x 10^{13} gc/kg. The subjects will be monitored for tolerance to the IMP and detection of immediate AEs for 24 hours (overnight stay) after dosing. The dosing of the subjects must be separated by a minimum of 14 calendar days to allow for subject safety monitoring and to ensure appropriate action can be taken in case any acute reactions are observed.

Trial Design/Methodology:

This is an open-label, single-dose, single-arm, multi-center trial, with a screening, a treatment + post-treatment follow-up phase, and a long-term follow-up phase.

Screening

Maximal six weeks prior to baseline subjects are screened for eligibility, and historical bleeding and factor IX use data will be collected. The subjects receive their electronic diary, and the Investigator/study nurse will train them in recording of the bleeding episodes and use of factor IX replacement therapy. From screening onwards, subjects will record their use of factor IX replacement therapy and bleeding episodes in this dedicated e-diary. Diary data will be reviewed on a continuous basis by the Investigator/study nurse. The period from screening up to baseline is considered a training period, after which the Investigator/study nurse will review and evaluate any problems with recording of e-diary data with the subject. The e-diary training can be repeated at any time during the trial as considered necessary by Investigator/study nurse. Baseline is defined as Day 0 prior to IMP administration (pre IMP).

The screening period might be prolonged in consultation with the sponsor in case IMP administration needs to be delayed because of subjects experiencing a bleeding episode, surgery, or other event after screening which warrants this delay.

IMP administration + Post-treatment follow-up phase

At baseline (i.e., Day 0 pre IMP), eligibility will be confirmed. It is recommended that subjects complete the Patient Reported Outcomes (PROs) prior to other assessments. Baseline samples will be taken for safety and efficacy assessments, physical examination will be performed and CCI will be assessed.

Once all baseline assessments have been performed and eligibility is confirmed, subjects will receive a single infusion of AMT-061. After IMP administration (post IMP), 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).

At baseline, subjects will be administered a challenge dose of short-acting factor IX (40 IU/kg) for the factor IX recovery assessment. This challenge dose should provide subjects sufficient factor IX coverage for the initial two to three days post treatment. Three days after IMP administration, the subjects should visit the clinic for endogenous factor IX activity assessment. If the factor IX activity result is ≥5%, further factor IX prophylaxis will not be given. If the factor IX activity result is <5%, another dose of short-acting factor IX will be administered to the subject and endogenous factor IX activity assessment will be performed again at the Week 1 routine visit. In a previous trial with AMT-060, therapeutic levels of factor IX protein and resultant factor IX activity levels were seen as early as one week after treatment.

Additional on-demand factor IX may be given after treatment with AMT-061, if considered necessary. If the endogenous factor IX activity ≥5%, factor IX prophylaxis will not be given and further management will be based on Investigator's clinical judgement and subject preference. Re-initiation of factor IX continuous routine prophylaxis may be considered if the endogenous factor IX activity is between 2% and 5% in at least two consecutive laboratory measurements, based on the Investigator's clinical judgment and subject preference. If endogenous factor IX activity is below 2% in at least two consecutive central lab measurements, continuous routine factor IX prophylaxis may be restarted.

From baseline until Week 52, subjects continue recording their use of factor IX replacement therapy and bleeding episodes in the e-diary. Diary data will be reviewed on a continuous basis by the Investigator/study nurse. In addition to the subjects' reporting of presumed bleeding episodes in the e-diary, the Investigator or designee will assess each bleeding episode, by describing the circumstances as well as the nature of the reported bleed in a bleed specific narrative as soon as possible but at least within 72 hours after it has been reported by the subject. In case the information provided in the e-diary is not sufficient to assess the presumed bleeding and describe it in a narrative, the subject needs to be called and/or visit the site. Further samples are taken for safety and efficacy laboratory parameters, physical examination will be performed, CCI will be assessed and subjects will complete the CCI. Use of concomitant medication and occurrence of AEs will be continuously monitored.

For ALT level increments of at least 2-fold baseline (i.e., pre-IMP), and/or greater than the upper limit of normal (ULN), by local or central laboratory, the Investigator should contact the Medpace and uniQure Medical Directors to discuss a clinical management plan on a case-by-case basis, including potential re-tests and/or initiation of corticosteroid treatment. In case of aspartate aminotransaminase (AST) level increments > ULN, the Investigator should contact the Medpace and uniQure Medical Directors and a similar discussion should take place.

Subjects are followed for a total duration of 52 weeks, with weekly visits for the first 12 weeks, every 2nd week from Week 12 to Week 26, and every month from Week 26 to Week 52.

One interim analysis will be performed after six weeks post-dose to determine the efficacy of AMT-061 in terms of factor IX activity. After 52 weeks post-dose and 2.5 years post-dose, the complete efficacy and safety data will be collected, locked, analyzed and reported in a Clinical Study Report (CSR) and a CSR addendum, respectively. At the end of the trial, all safety and efficacy data will be reported in a CSR addendum (5-year analysis).

Long-term follow-up phase

After post-treatment follow-up, subjects will enter a long-term follow-up phase for an additional four years to assess sustainability of efficacy and long-term safety. During this phase, subjects do not have to record e-diary data, but are expected to document factor IX usage and bleeding episode information in study-specific paper diaries. Subjects are expected to bring their long-term follow-up bleed diaries and long-term follow-up factor IX use diaries to every study visit during the long-term follow-up phase, and site staff will collect the new information at each visit. In between study visits, subjects should contact the site staff immediately in case of an experienced bleed and/or factor IX use in addition to completing the questions/information requested on the paper diaries to capture all information. Subjects will visit the clinic every half year for evaluation of efficacy parameters and safety. Beginning at the Month 30 visit, and at every visit thereafter (occurring every 6 months) until study completion, an abdominal ultrasound will be performed. Occurrence of AEs will be continuously monitored, with at least quarterly contact moments between site staff and subject to discuss. 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, via the site staff, as much as possible of changes to the study and monitoring plans that could impact them.

Please refer to Table 1 through Table 4 for an overview of the assessments and testing performed throughout these different trial phases.

Trial Assessments:

Efficacy assessments include the e-diary for recording of bleedings and factor IX use, several laboratory parameters (including factor IX protein and activity levels), assessment of CCI, and the CCI on general and disease specific CCI.

Safety is assessed by AEs, physical examination, and laboratory parameters.

Inclusion and Exclusion Criteria:

Inclusion Criteria:

1. Male
2. Age ≥18 years
3. Subjects with congenital hemophilia B classified as one of the following:
   a. Known severe factor IX deficiency (<1% of normal circulating factor IX) for which the subject is either on continuous routine factor IX prophylaxis* or using on-demand factor IX replacement therapy
   b. Known moderately severe factor IX deficiency (1-2% of normal circulating factor IX, inclusive) and a severe bleeding phenotype as defined by at least one of the following:
      i. On continuous routine factor IX prophylaxis* for a history of bleeding
      ii. On demand factor IX replacement therapy with a history of frequent bleeding (4 or more bleeding episodes in the last 12 months) or chronic hemophilic arthropathy (pain, joint destruction, and loss of range of motion) in one or more joints
4. >20 previous exposure days of treatment with factor IX 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 three consecutively collected semen samples (this criterion is applicable also for subjects who are surgically sterilized)
6. Able to provide informed consent following receipt of verbal and written information about the trial.

** Continuous routine prophylaxis is defined as the intent of treating with an a priori defined frequency of infusions (e.g., twice weekly, once every two weeks, etc.) as documented in the medical records*

Exclusion Criteria:

1. History of factor IX inhibitors
2. Positive factor IX inhibitor test at screening
3. Screening laboratory values:
   a. ALT >2 times ULN
   b. AST >2 times ULN
   c. Total bilirubin >2 times ULN
   d. Alkaline phosphatase (ALP) >2 times ULN
   e. Creatinine >2 times ULN
4. Positive human immunodeficiency virus (HIV) serological test at screening, not controlled with anti-viral therapy as shown by CD4+ counts ≤200/µL or by a viral load of >200 copies/mL
5. 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
6. History of hepatitis B or C exposure, currently controlled by antiviral therapy

7. Known coagulation disorder other than hemophilia B
8. Thrombocytopenia, defined as a platelet count below 50×10^9/L, at screening
9. Known severe infection or any other significant concurrent, uncontrolled medical condition including, but not limited to, renal, hepatic, cardiovascular, hematological, 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
10. Known significant medical condition that may significantly impact the intended transduction of the vector and/or expression and activity of the protein, such as disseminated intravascular coagulation, accelerated fibrinolysis, and profound liver fibrosis
11. Known history of an allergic reaction or anaphylaxis to factor IX products
12. Known uncontrolled allergic conditions or allergy/hypersensitivity to any component of the IMP excipients
13. Known medical condition that would require chronic administration of steroids
14. Previous gene therapy treatment
15. Receipt of an experimental agent within 60 days prior to screening
16. Current participation or anticipated participation within one year after IMP administration in this trial in any other interventional clinical trial involving drugs or devices

Maximum Duration of Subject Involvement in the Trial:

- Planned duration of screening period: maximal six weeks
- Planned duration of treatment: 1 day (single dose)
- Planned duration of follow-up: 52 weeks, plus long-term safety follow-up until Month 60

Endpoints:

The primary aim of the trial is to demonstrate that AMT-061 will provide a minimal therapeutic response of ≥5% factor IX activity using a dose of 2×10^{13} gc/kg. This is the proposed dose to be used in a further pivotal Phase 3 trial. The primary efficacy parameter is factor IX activity level at six weeks after dosing (at Week 6 post AMT-061 dose).

Secondary efficacy endpoints comprise of endogenous factor IX activity level at Week 6 and Week 52 post AMT-061 dose, remaining free of previous continuous routine prophylaxis during 52 weeks following AMT-061 dosing, total usage of factor IX replacement therapy until 52 weeks following AMT-061 dosing (excluding ad hoc prophylaxis for invasive procedures), and annualized bleeding rate after 52 weeks of AMT-061 dosing (including a further break down of the frequency and percentage of spontaneous, traumatic, and joint bleeding events).

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Secondary safety endpoints include AEs, hematology and serum chemistry parameters, ALT/AST levels and corticosteroid use for ALT/AST elevations, parameters on antibody formation to AAV5 and human factor IX, AAV5 capsid-specific T cell response, inflammatory markers, vector DNA in semen and blood, and alpha-fetoprotein (AFP). Safety endpoints are observed over the 52-week post-treatment follow-up phase and for an additional four years in the long-term follow-up phase.

Statistical Analysis:

There is no formal sample size calculation performed for this trial. From a clinical perspective, N=3 subjects is considered sufficient to provide a reliable impression on the factor IX activity levels that will be demonstrated with a single dose of 2×10^{13} gc/kg AMT-061 at six weeks after dosing.

Considering the small sample size used in this trial, all presentations will be descriptive in nature. Plots, tabular displays, and listings will be created, visualizing individual effects for selected efficacy and safety measures. If applicable, continuous variables will be summarized with descriptive statistics including: the number of non-missing values, mean, standard deviation, median, minimum, and maximum. In some cases, the standard error of the mean and/or confidence intervals may be provided as well. Categorical variables will be summarized by

number, percent of subjects, and if applicable, the number of events. No formal inferential statistical analyses will be performed, and no analysis population will be defined. All available data will be used in the presentations.

Analyses will be based on central laboratory measurements if results are available from both local and central laboratories.

After six weeks post-dose, an interim analysis for efficacy will be done using the available data on factor IX activity and safety data will be evaluated. The results will be reported in an interim CSR. The rationale for the six weeks cut-off is based on data from the Phase 1/2 trial that demonstrated a very fast initial transduction of AAV5 and expression of the endogenous factor IX protein within the first week that reached a relatively stable peak level within the first six weeks of follow-up.

After 52 weeks post-dose and 2.5 years post-dose, efficacy and safety data will be collected on all subjects. Those data will be locked, analyzed, and reported in a CSR (52-week analysis) and CSR addendum (2.5-year analysis). At the end of the 4-year long-term follow-up period (60 months/5 years post-dose), efficacy and safety data will be collected on all subjects. The data will be locked, analyzed, and reported in a CSR addendum (5-year analysis).

Trial Schedules

Table 1 Flow Chart for Efficacy and Safety Evaluation, for Screening, IMP Administration + Post-Treatment Follow-up (Week -6 to Week 52)

Trial Period	**Screening**	**IMP Administration**			**Post-treatment Follow-up**						**Additional Visits** [a]
Weeks/Day	W-6	D0 pre IMP [b]	D0 0h	D0 post IMP	D1	D3	W1 to 12 [b, c] (1 visit/W)	W14 to 26 [b, c] (every 2nd W)	W31 to 48 [b, c] (1 visit/month)	W52 [b, c]	
Visit Window (Days)	0	0	0	0	0	±1	±2	±3	±5	±5	
Informed consent	x										
In-/exclusion criteria	x	x									
Medical history	x	x									
Collection of historical bleeding and factor IX use data	x										
24-hr stay at clinic		x----------x									
Administration of IMP			x								
Discharge and hand-out of subject treatment card [d]					x						
Concomitant medication	Continuous										(x) [a]
Vital signs (blood pressure, pulse, body temperature)	x	x		x [h]	x [h]						(x) [a]
Physical examination	x	x					x	x	x	x	(x) [a]
Height	x										
Body weight	x										(x) [a]
Blood & semen sampling (see Table 2)	x	x		x	x	x	x	x	x	x	(x) [a]
Subject hand-out e-diary device and e-diary instruction [e]	x										
Recording of e-diary data by subject (bleeding episodes, factor IX replacement therapy)	Continuous										
Review of e-diary data by Investigator/study nurse	Continuous										(x) [a]

Trial Period	Screening	IMP Administration			Post-treatment Follow-up						Additional Visits [a]
Weeks/Day	W-6	D0 pre IMP [b]	D0 0h	D0 post IMP	D1	D3	W1 to 12 [b, c] (1 visit/W)	W14 to 26 [b, c] (every 2nd W)	W31 to 48 [b, c] (1 visit/month)	W52 [b, c]	
Visit Window (Days)	0	0	0	0	0	±1	±2	±3	±5	±5	
Collection of e-diary device										x	
Subjects provided with paper diaries and paper diary instruction for Long-term Follow-up Phase										x	
Adjudication of bleeding episode(s) by Investigator[f]	Continuous										(x) [a]
CCI		x						x (w26)		x	
CCI		x						x (w26)		x	
CCI		x						x (w26)		x	
CCI		x						x (w26)		x	
CCI		x						x (w26)		x	
CCI		x								x	
Adverse events	Continuous										(x) [a]

CCI IMP: investigational medicinal product; ; W: week.

a. An additional visit can be performed at any time for the purpose of conduct of 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.
b. For subjects on routine prophylactic factor IX replacement therapy, these visits should take place on the day that routine prophylactic factor IX replacement treatment is planned to be administered. At these visits, blood sampling will then take place prior to administration of prophylactic factor IX replacement therapy. If a subject uses additional on-demand factor IX replacement treatment, his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit takes place at the time the subject resumes his routine prophylaxis schedule. For subjects only using on-demand factor IX replacement therapy (only applicable after AMT-061 administration), his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit does not take place within 10 days of any on-demand factor IX product use.
c. Each visit is scheduled in relation to date of IMP administration, not in relation to the date of its previous visit.
d. When all assessments at 24 hours after IMP administration are performed, the subject treatment card will be handed out and the subject may leave the clinic.
e. Instruction can be repeated at any visit as deemed necessary by the Investigator or study nurse.
f. The Investigator or designee will assess each bleeding episode, by describing the circumstances as well as the nature of the reported bleed in a bleed specific narrative as soon as possible but at least within 72 hours after it has been reported by the subject.
g. It is recommended that the CCI (CCI) are completed by the subject, before he is seen by the Investigator and/or study nurse for interview and before other (trial specific) assessments are performed, as well as before the administration of IMP, in order for the answers not to be influenced by the information given by the physician, by the administration of IMP, or by early side-effects of the IMP or (trial related) assessments. At each visit, the questionnaires are to be completed in the same order every time, following the order presented in the protocol.
h. Vital signs (blood pressure, pulse, body temperature) will be measured at t=0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours after the IMP infusion has been completed.

Table 2 Flow Chart for Laboratory Parameters, for Screening, IMP Administration + Post-Treatment Follow-up (Week -6 to Week 52)

Trial Period	**Screening**	**IMP Administration**				**Post-treatment Follow-up**						**Additional Visits** [a]
Weeks/Day	W-6	D0 pre IMP [b]	D0 post IMP			D1	D3	W1 to 12 [b, c] (1 visit/W)	W14 to 26 [b, c] (every 2nd W)	W31 to 48 [b, c] (1 visit/month)	W52 [b, c]	
Hours After IMP Infusion Completed			1h	2h	3h	24h						
Visit Window (Days)			±15 minutes			±15 minutes	±1	±2	±3	±5	±5	
Local Laboratory												
Factor IX: one-stage aPTT for factor IX activity for local monitoring		x					x	x	x	x	x	(x) [a]
Factor IX inhibitors (Bethesda assay or Nijmegen modified Bethesda assay) for local monitoring and eligibility check [d]	x	x						x: W6, 12	x: W26		x	(x) [a]
Transaminases (AST/ ALT) for local monitoring [e]	x	x						x	x	x	x	(x) [a]
Central Laboratory												
One-stage aPTT for factor IX activity		x						x	x	x	x	(x) [a]
Chromogenic assay for factor IX activity [f]		x						x	x	x	x	(x) [a]
Factor IX protein concentration		x						x	x	x	x	(x) [a]
Anti-factor IX antibodies		x						x: W6, 12	x: W26		x	(x) [a]
Factor IX inhibitors (Nijmegen modified Bethesda assay) [d]	x	x						x: W6, 12	x: W26		x	(x) [a]
Factor IX Recovery [g]		x										(x) [a]
Total (IgM and IgG) antibodies to AAV5	x	x						x: W1, 2, 3, 4, 5, 6, 12	x: W26		x	(x) [a]
Neutralizing antibodies to AAV5	x	x						x: W1, 2, 3, 4, 5, 6, 12	x: W26		x	(x) [a]
AAV5 capsid-specific T cells		x						x	x	x	x	(x) [a]

Trial Period	**Screening**	**IMP Administration**				**Post-treatment Follow-up**						**Additional Visits** [a]
Weeks/Day	W-6	D0 pre IMP [b]	D0 post IMP			D1	D3	W1 to 12 [b, c] (1 visit/W)	W14 to 26 [b, c] (every 2nd W)	W31 to 48 [b, c] (1 visit/month)	W52 [b, c]	
Hours After IMP Infusion Completed			1h	2h	3h	24h						
Visit Window (Days)			±15 minutes			±15 minutes	±1	±2	±3	±5	±5	
Sampling for vector genome detection:												
- Blood [h]		x	x	x	x	x		x	x	x	x	(x) [a]
- Semen [h]		x						x:W6, 12	x:W16, 26		x	(x) [a]
Inflammatory markers IL-1β, IL-2, IL-6, IFNγ, MCP-1		x						x	x	x	x	(x) [a]
Alpha-fetoprotein											x	(x) [a]
Hematology and coagulation parameters [i]	x	x						x	x	x	x	(x) [a]
Serum chemistry parameters [j]	x	x			x (only CRP)			x	x	x	x	(x) [a]
HIV, viral load, $CD4^+$	x											
HBsAg, HBeAg, HBV DNA and HCV RNA	x											
Factor IX gene sequencing [k]	x											
Blood sample for future research [l]	x	x						x: W12			x	

AAV5: adeno-associated viral vector serotype 5; ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; CRP: C-Reactive Protein; DNA: deoxyribonucleic acid; h: hour; HBeAg: hepatitis B extracellular antigen; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HCV RNA: hepatitis C virus ribonucleic acid; HIV: human immunodeficiency virus; IFNγ: interferon gamma; IgG: immunoglobulin G; IgM: immunoglobulin M; IL: interleukin: IMP: investigational medicinal product; MCP-1: monocyte chemotactic protein-1.

a. An additional visit can be performed at any time for the purpose of conduct of 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.
b. For subjects on routine prophylactic factor IX replacement therapy, these visits should take place on the day that routine prophylactic factor IX replacement treatment is planned to be administered. At these visits, blood sampling will then take place prior to administration of prophylactic factor IX replacement therapy. If a subject uses additional on-demand factor IX replacement treatment, his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit takes place at the time the subject resumes his routine prophylaxis schedule. For subjects only using on-demand factor IX replacement therapy (only applicable after AMT-061 administration), his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit does not take place within 10 days of any on-demand factor IX product use.
c. 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.
d. In case of a positive test for factor IX inhibitors, a re-test should be performed with two weeks to confirm the positive test. The subject should be called in for an additional visit in case no routine visit is scheduled within this two-week timeframe.

e. For ALT level increments of at least 2-fold baseline (i.e., pre-IMP) and/or > upper limit of normal (ULN), by local or central laboratory, the Investigator should contact the Medpace and uniQure Medical Directors to discuss a clinical management plan, on a case-by-case basis, including potential re-tests and/or initiation of corticosteroid treatment. In case of AST level increments > ULN, the Investigator should contact the Medpace and uniQure Medical Directors and a similar discussion should take place.
f. No additional blood will be drawn for the chromogenic assay; the test will be performed on backup samples.
g. At suspicion of factor IX inhibitor, the factor IX recovery assessment should be repeated. Blood samples for factor IX recovery assessment should be drawn just prior to administering the factor IX challenge dose (40 U/kg) and at 30 minutes after the factor IX challenge dose is administered.
h. Sampling for the individual subject and for a specific matrix (i.e., blood or semen) is only to be continued until 3 consecutive negative samples have been detected for the subject for that particular type of matrix. The sampling schedule (frequency) may be increased as agreed between the Investigator and subject following the notification of a first negative result on blood and/or semen, expediting the opportunity to reach three consecutive negative samples on the specific matrix. Based on the wish of the subject, semen samples can be collected at home prior to attending the visit (at the visit day or at the day before the visit day). Also, the frequency of semen sampling may be reduced (to be agreed between Investigator and subject) as long as the subject uses a condom during sexual intercourse until 3 consecutive negative samples have been detected.
i. For hematology: Hemoglobin, hematocrit, platelet count, red blood cells, white blood cells with differential count (all expressed in % as well as in absolute numbers); for coagulation: aPTT and prothrombin time (or INR [International Normalized Ratio])).
j. Serum electrolytes (sodium, potassium), creatinine, creatine kinase, gamma-glutamyltransferase (γGT), AST, ALT, alkaline phosphatase (ALP), CRP, albumin, total bilirubin, glucose (non-fasting).
k. Only if factor IX gene mutation information is not available and if separate informed consent for factor IX gene sequencing analysis is given by the subject. Preferably at the screening visit, but otherwise at a later time point during the subject's trial participation.
l. Only if separate informed consent is given by the subject.

Table 3 Flow Chart for Efficacy and Safety Evaluation, for Long-Term Follow-up (up to Month 60)

Trial Period	Long-Term Follow-up								Additional Visits [a]
Months	18 [b, c]	24 [b, c]	30 [b, c]	36 [b, c]	42 [b, c]	48 [b, c]	54 [b, c]	60 [b, c]	
Visit Window (Weeks)	±2	±2	±2	±2	±2	±2	±2	±2	
Concomitant medication	Continuous								(x) [a]
Collection of bleeding and factor IX use data [d]	x	x	x	x	x	x	x	x	(x) [a]
Physical examination	x	x	x	x	x	x	x	x	(x) [a]
Blood & semen sampling (see Table 4)	x	x	x	x	x	x	x	x	(x) [a]
CCI		x		x		x		x	
CCI		x		x		x		x	
CCI		x		x		x		x	
CCI		x		x		x		x	
CCI		x		x		x		x	
CCI		x		x		x		x	
Abdominal ultrasound [f]			x	x	x	x	x	x	(x) [a]
Adverse events [g]	Continuous								

CCI ; IMP: investigational medicinal product; CCI

a. An additional visit can be performed at any time for the purpose of conduct of 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.
b. 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.
c. For subjects on routine prophylactic factor IX replacement therapy, these visits should take place on the day that routine prophylactic factor IX replacement treatment is planned to be administered. At these visits, blood sampling will then take place prior to administration of prophylactic factor IX replacement therapy. If a subject uses additional on-demand factor IX replacement treatment, his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit takes place at the time the subject resumes his routine prophylaxis schedule. For subjects only using on-demand factor IX replacement therapy (only applicable after AMT-061 administration), his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit does not take place within 10 days of any on-demand factor IX product use.
d. Subjects will record bleeding episodes and factor IX replacement therapy use in their study-specific paper long-term follow-up bleed diary and paper long-term follow-up factor IX use diary, which they will bring to every study visit. Site staff will collect the information that is new since the previous visit in the paper diaries. In between study visits, subjects should contact the site staff immediately in case of an experienced bleed and/or factor IX use in addition to completing the questions/information requested on the paper diaries.
e. It is recommended that the CCI (CCI) are completed by the subject before he is seen by the physician for interview and before other (trial specific) assessments are performed, as well as before the administration of IMP, in order for the answers not to be influenced by the information given by the physician, by the administration of IMP, or by early side-effects of the IMP or (trial related) assessments. During the screening visit, the CCI are completed after ICF signature, but before other (trial specific) assessments are performed. At each visit, the questionnaires are to be completed in the same order every time, following the order presented in the protocol. The questionnaires are to be completed under site supervision at the site. For those subjects where the Month 24, Month 36, Month 48, and/or Month 60 visits are impacted by COVID-19, the questionnaires may be conducted at the site within the following window: up to -1 month prior to the target visit, up to +2 months after the target visit, and at least 4 months after the last assessment. Adjustments to the visit timing within this window are to be documented.
f. For those subjects where Month 30, Month 36, Month 42, Month 48, Month 54, and/or Month 60 visits are impacted by COVID-19, abdominal ultrasounds may be conducted within the following window: up to -1 month prior to the target visit and up to +1 month after the target visit. Adjustments to the visit timing within this window are to be documented.
g. After week 52, the visit frequency is every 26 weeks (6 months). In this long-term follow-up phase there should be at least quarterly contact between the site staff and subject to monitor the occurrence of adverse events.

Table 4 Flow Chart for Laboratory Parameters, for Long-Term Follow-up (up to Month 60)

Trial Period	Long-Term Follow-up								Additional Visits [a]
Months	18 [b, c]	24 [b, c]	30 [b, c]	36 [b, c]	42 [b, c]	48 [bb, c]	54 [b, c]	60 [b, c]	
Visit Window (Weeks)	±2	±2	±2	±2	±2	±2	±2	±2	
Local Laboratory									
Factor IX: one-stage aPTT for factor IX activity for local monitoring			x	x	x	x	x	x	(x) [a]
Transaminases (AST/ ALT) for local monitoring [d]			x	x	x	x	x	x	(x) [a]
Central Laboratory									
One-stage aPTT for factor IX activity	x	x	x	x	x	x	x	x	(x) [a]
Chromogenic assay for factor IX activity [e]	x	x	x	x	x	x	x	x	(x) [a]
Factor IX protein concentration	x	x	x	x	x	x	x	x	(x) [a]
Anti-factor IX antibodies		x		x		x		x	(x) [a]
Factor IX inhibitors (Nijmegen modified Bethesda assay) [f]		x		x		x		x	(x) [a]
factor IX Recovery [g]									(x) [a]
Total (IgM and IgG) antibodies to AAV5		x		x		x		x	(x) [a]
Neutralizing antibodies to AAV5		x		x		x		x	(x) [a]
Sampling for vector genome detection:									
- Blood [h]	x	x	x	x	x	x	x	x	(x) [a]
- Semen [h]	x	x	x	x	x	x	x	x	(x) [a]
Alpha-fetoprotein	x	x	x	x	x	x	x	x	(x) [a]
Hematology and coagulation parameters [i]	x	x	x	x	x	x	x	x	(x) [a]
Serum chemistry parameters [j]	x	x	x	x	x	x	x	x	(x) [a]

AAV5: adeno-associated viral vector serotype 5; ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; IgG: immunoglobulin G; IgM: immunoglobulin M; IMP: investigational medicinal product.

a. An additional visit can be performed at any time for the purpose of conduct of 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.

b. 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.

c. For subjects on routine prophylactic factor IX replacement therapy, these visits should take place on the day that routine prophylactic factor IX replacement treatment is planned to be administered. At these visits, blood sampling will then take place prior to administration of prophylactic factor IX replacement therapy. If a subject uses additional on-demand factor IX replacement treatment, his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit takes place at the time the subject resumes his routine prophylaxis schedule. For subjects only using on-demand factor IX replacement therapy (only applicable after AMT-061

administration), his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit does not take place within 10 days of any on-demand factor IX product use.

d. For ALT level increments of at least 2-fold baseline (i.e., pre-IMP) and/or > upper limit of normal (ULN), by local or central laboratory, the Investigator should contact the Medpace and uniQure Medical Directors to discuss a clinical management plan, on a case-by-case basis, including potential re-tests and/or initiation of corticosteroid treatment. In case of AST level increments > ULN, the Investigator should contact the Medpace and uniQure Medical Directors and a similar discussion should take place.
e. No additional blood will be drawn for the chromogenic assay; the test will be performed on backup samples.
f. In case of a positive test for factor IX inhibitors, a re-test should be performed with two weeks to confirm the positive test. The subject should be called in for an additional visit in case no routine visit is scheduled within this two-week timeframe.
g. At suspicion of factor IX inhibitor, the factor IX recovery assessment should be repeated. Blood samples for factor IX recovery assessment should be drawn just prior to administering the factor IX challenge dose (40 U/kg) and at 30 minutes after the factor IX challenge dose is administered.
h. Sampling for the individual subject and for a specific matrix (i.e., blood or semen) is only to be continued until 3 consecutive negative samples have been detected for the subject for that particular type of matrix. The sampling schedule (frequency) may be increased as agreed between the Investigator and subject following the notification of a first negative result on blood and/or semen, expediting the opportunity to reach three consecutive negative samples on the specific matrix. Based on the wish of the subject, semen samples can be collected at home prior to attending the visit (at the visit day or at the day before the visit day). Also, the frequency of semen sampling may be reduced (to be agreed between Investigator and subject) as long as the subject uses a condom during sexual intercourse until 3 consecutive negative samples have been detected. Semen samples are only to be collected as applicable.
i. For hematology: hemoglobin, hematocrit, platelet count, red blood cells, white blood cells with differential count (all expressed in % as well as in absolute numbers); for coagulation: aPTT and prothrombin time (or INR [International Normalized Ratio]).
j. Serum electrolytes (sodium, potassium), creatinine, creatine kinase, gamma-glutamyltransferase (γGT), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), C-Reactive Protein (CRP), albumin, total bilirubin, glucose (non-fasting).

1 INTRODUCTION

1.1 Condition Background and Current Treatment

Congenital hemophilia B is an inherited bleeding disorder characterized by an increased bleeding tendency due to either a partial or complete deficiency of the essential blood coagulation factor IX (FIX). The deficiency is the result of mutations of the respective clotting factor genes. Hemophilia B is an X-linked, recessive condition, since it occurs almost exclusively in males. Females typically are asymptomatic carriers. The number of people with hemophilia B worldwide is approximately 30,000 and in the United States (US) alone is approximately 4,000 (World Federation of Haemophilia [WFH], 2017). Approximately 1 in 20,000 - 50,000 live male newborns has hemophilia B.

The severity of symptoms can vary and the severe forms become apparent early in life. About one-third of individuals with hemophilia B have a severe disorder characterized by functional factor IX levels that are less than 1% of normal (Kessler & Mariani, 2006). Moderate and mild hemophilia B, with 1 - 5% or 5 - <40% of normal factor IX level, respectively, are each observed in about one-third of patients (Kessler & Mariani, 2006).

Bleeding is the main symptom of the disease and usually increases when the infant becomes mobile. Mild cases may go unnoticed until later in life, when they occur in response to surgery or trauma. In severe or moderate hemophilia internal bleeding may occur anywhere, but bleeding into joints is most common (National Heart, Lung, and Blood Institute, 2013). Severe recurrent bleedings may lead to severe arthropathy, joint contractures and pseudotumors, resulting in chronic pain, disability and reduced CCI (Bolton-Maggs et al., 2003). As few as one to two bleeds can trigger progressive, irreversible joint disease (Gater et al., 2011).

The overall life expectancy in severe hemophilia is 63 years, which is about 15 years lower than general male population (Darby et al., 2007). Life expectancy in patients without liver complications (e.g., human immunodeficiency virus [HIV] or hepatitis C) can approach that of the general male population (Plug et al, 2006; Osooli et al 2017).

There is no cure for hemophilia B. The primary goals of hemophilia B therapy are the prevention of bleeding episodes, rapid and definitive treatment of bleeding episodes (breakthrough bleeds) that occur even while on a regular prophylactic regimen and provision of adequate hemostasis during surgery and emergencies. Currently, these goals are essentially met for hemophilia B subjects by intravenous (IV) injections of commercially available recombinant- or plasma-derived factor IX products, either at the time of a bleed (on-demand) or by regular infusions up to several times a week (prophylactically). The recent approvals of extended half-life factor IX products allow for reduced frequency of factor administration (once every 7 to 21 days) and maintaining a higher factor IX trough level (Taylor and Kruse-Jarres, 2016).

1.2 Gene Therapy Therapeutic Concept

Somatic gene therapy offers the potential for a shift of the disease severity phenotype from severe to a moderate or mild hemophilia phenotype or complete amelioration through continuous production of stable factor IX levels after a single administration of vector, especially since a small rise in circulating factor IX to at least 1% of normal levels can substantially ameliorate the bleeding phenotype and thus improve the CCI for patients.

1.3 Investigational Product Background

The company has developed AMT-060, a recombinant adeno-associated viral vector serotype 5 (AAV5) containing the wild-type human factor IX (hFIX) gene, codon-optimized for optimal expression in humans, under control of a liver-specific promoter (AAV5-hFIXco or AMT-060). AMT-060 has been studied in a Phase 1/2 clinical trial. The investigational medicinal product (IMP) that will be used in the current protocol is a derivative of AMT-060: the same recombinant AAV5 containing the codon-optimized wild-type human factor IX gene, incorporating a two-nucleotide change in order to express the naturally occurring Padua variant of human coagulation factor IX complementary DNA (cDNA), i.e., AMT-061 or AAV5-hFIXco-Padua. The FIX-Padua protein differs from the 'wild-type' human factor IX protein by a single amino acid and possesses a specific activity approximately 6-fold higher than wild-type.

This is a first-in-human clinical trial for AMT-061. Both AMT-060 and AMT-061 employ the same AAV5 capsid as vector and liver-specific promoter, conferring similar safety and expression profiles. Section 1.3.1 provides a summary of the preclinical information for AMT-060 and AMT-061. Clinical information for AMT-060 is summarized in Section 1.3.2. For the most comprehensive information regarding AMT-060 and AMT-061 refer to the most recent Investigator's Brochure.

1.3.1 Preclinical Information

Information for AMT-060

A range of *in vivo* studies in wild-type and hemophilia B mice and in non-human primates (NHPs) have been performed to characterize the safety and pharmacology of AMT-060.

Pharmacology studies in mice and NHPs showed out that infusion of AMT-060 resulted in vector dose-dependent circulating levels of (human) factor IX protein.

To verify that AMT-060 mediates expression of biologically active human factor IX, and to confirm that the human factor IX produced and secreted into the circulation can ameliorate the clotting defect inherent to factor IX deficiency, a dose-range study was performed in a murine model of hemophilia B (Study NR-060-13-007). Results of this study indicated direct correlation *in vivo* between circulating human factor IX protein levels and human factor IX activity.

In both Rhesus and Cynomolgus macaques injected with various doses of AMT-060, the resulting human factor IX expression correlated with the dose and was sustained for the duration of the 6 months follow-up (Cynomolgus macaque; Study 522156 and NR-060-14-006) and 90 days follow-up (Rhesus monkey; Study 520665 and NR-060-11-009).

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.

These non-clinical data suggest that IV administration of AMT-060 is able to mediate sustained levels of factor IX, and that such administration is not associated with any significant safety concerns.

Information for AMT-061

The AMT-060 and AMT-061 based gene therapy vectors are identical with the exception of a two-nucleotide substitution resulting in a single codon change (AGG to CTG) in the coding sequence for factor IX, corresponding to an Arginine to Leucine substitution in the transgenic protein.

The R338L substitution, FIX-Padua, represents a naturally occurring variant of factor IX showing a gain-of-function, which results in higher factor IX activity with similar factor IX protein expression (Simioni et al., 2009). This augmentation is thought to be largely caused by increased affinity of the activated protein to activated clotting factor VIII (FVIIIa; Kao et al., 2013). Both AMT-060 and AMT-061 employ the same vector AAV5 without any changes and it is therefore expected that AMT-061 will present a similar safety profile.

A NHP study (NR-061-17-001) was performed to assess 4 different dose levels of AMT-061 in direct comparison with AMT-060 with respect to liver transduction, circulating factor IX protein levels, circulating factor IX activity levels and toxicity, after a single IV dose with 13 or 26-week observation period. The study comprised of measurements of circulating human factor IX protein levels, total circulating factor IX activity levels, assessment of vector deoxyribonucleic acid (DNA) in plasma, biodistribution including more than 25 different organs/tissues, complete safety panel routinely performed in GLP-toxicity studies and monitoring of six different liver enzymes and additional coagulation markers throughout the study. The study revealed dose dependent plasma vector DNA levels, human factor IX protein levels and factor IX activity for AMT-061. Clinical signs were unaffected by treatment as well as clinical chemistry and hematology. Coagulation investigations up to 26 weeks revealed a shortening of the activated partial thromboplastin time (aPTT) and longer prothrombin time (PT) clotting times at the highest dose (9.0×10^{13} genome copies [gc]/kg) of AMT-061 only, with unaffected plasma D-dimer and thrombin-antithrombin levels, indicating some effect on the clotting cascade within physiological compensating boundaries and without an increase of thrombin utilization. The study clearly showed that at a dose of 5×10^{12} gc/kg, plasma exposure, liver distribution, liver cell transduction and transgene expression are similar for both AMT-060 and AMT-061 and that

transgene activity is approximately 6 times higher per unit protein for AMT-061 as compared to AMT-060. Although the study design only included a direct comparison at 5 x 10^{12} gc/kg, it can be expected that plasma exposure, liver distribution, liver cell transduction and transgene expression behave similarly at each dose level with a range. As expected, the study demonstrated that for the same dose, the mean factor IX protein of AMT-060 (average of 4.89, ranging from 3.17 to 7.61 %) and AMT-061 (average of 4.85, ranging from 2.92 to 6.17 %) was comparable. The confirmation of transgene activity being approximately six times higher per unit protein (average of 6.10, ranging from 5.41 to 7.47 %) can similarly be expected to result in multiple of the already demonstrated activity levels of AMT-060 within a range. These results are comparable to the increase in gain-of-function reported for the Padua factor IX protein compared with "wild-type" factor IX protein in animal models (Crudele et al., 2015; Monahan et al., 2015).

The observed translation of the NHP for AMT-060 to the clinical Phase 1/2 for AMT-060 supports the translation of the NHP study for AMT-061 (NR-061-17-001, Study No. NC1615) to further inform the dose rationale for the proposed dose confirmation trial with 2 × 10^{13} gc/kg AMT-061. At 2 x 10^{13}gc/kg, AMT-061 is predicted to result in mean factor IX activity ranges of 40% of normal, with acceptable ranges (between approximately 18-76%) within the expected safety for factor IX replacement with an upper range well below the cut off of 129%.

1.3.2 Clinical Information for AMT-060 and Preliminary Information for AMT-061

The combination of an AAV5 vector and the human factor IX gene (AAV5-hFIX) had not been evaluated in human trials prior to the initiation of the Sponsor's Phase 1/2 clinical trial CT-AMT-060-01. The AAV5 capsid and the human factor IX gene cassette, as well as the hFIX-Padua cassette, have been tested individually in other Phase 1/2 clinical trials. The results from these trials providing preliminary clinical evidence for efficacy and safety of AMT-061 are summarized in Section 1.3.2.1, and the results of the first in human trial (CT-AMT-060-01) are summarized in Section 1.3.2.2.

1.3.2.1 Preliminary Clinical Evidence for Efficacy and Safety of Components of AMT-060

In Table 5 the most relevant published data and studies conducted with adeno-associated viral vector (AAV) capsids and human factor IX that were identified through searches of PubMed and clinicaltrials.gov databases are presented. The data outlined below support that administration of AMT-061 will be safe, well-tolerated, and able to generate sustained endogenous production of factor IX at clinically meaningful activity levels.

Table 5 Summary of Published Clinical Experience with Components of AMT-060

Reference	Capsid	Gene	Promotor	Target Tissue	Key Findings
D'Avola, 2016	AAV5	PBGD	--	Liver	- No adverse events associated with capsid - Capable of transducing hepatocytes *in vivo*
Pasi, 2016	AAV5	BDD-FVIII	--	Liver	- Mild asymptomatic transaminase elevations in 4 subjects - Capable of delivery that results in clinically relevant transgene expression
Lu et al, 1993	Retro-viral	hFIX	LTR N2CMV	Autologous skin fibroblasts	- Increase in factor IX activity and improved symptoms in 1 subject - No safety or toxicity concerns
Kay, 2000	AAV2	hFIX	CMV	Muscle	- Expression of biologically active human factor IX - No inhibitors to factor IX
Manno, 2003	AAV2	hFIX	CMV	Muscle	- Safe incorporation of human factor IX into muscle cells - Persistence of vector genome and protein expression to 10 months - No inhibitors to factor IX
Jiang, 2006	AAV2	hFIX	CMV	Muscle	- Persistence of vector genome and local protein expression at 3.7 years post treatment - No inhibitors to factor IX
Manno, 2006	AAV2	hFIX	hAAT-APOE	Liver	- Transient expression of factor IX at clinically relevant doses by transduced hepatocytes - Loss of factor IX expression with ALT elevation - No inhibitors to factor IX or T-cell response to the capsid
Nathwani, 2011	AAV8	hFIXco[a]	LP1[a]	Liver	- Successful transduction of hepatocytes resulting in clinically relevant increases in serum factor IX activity - Elevated transaminases in 4 subjects; factor IX expression retained in 2 with corticosteroid treatment - No inhibitors to factor IX
Nathwani, 2014	AAV8	hFIXco[a]	LP1[a]	Liver	- Clinically relevant increases in FIX activity sustained up to approximately 4.5 years - No inhibitors to factor IX - No late toxicity

Reference	Capsid	Gene	Promotor	Target Tissue	Key Findings
George, 2017	Undisclosed	hFIX-Padua	Undisclosed	Liver	- Clinically relevant increases in factor IX activity - Elevated transaminases in 2 subjects; partial to full factor IX activity retained with corticosteroid treatment - No inhibitors to factor IX

AAV5/AAV2/AAV8: adeno-associated viral vector of serotype 5 (or 2 or 8); ALT: alanine aminotransferase; APOE: apolipoprotein E; BDD-FVIII: B-domain deleted factor VIII; CMV: Cytomegalovirus; hAAT: human α1-antitrypsin; LTR: long terminal repeat; PBGD: porphobilinogen deaminase.
[a] Same transgene expression cassette as is used for AMT-060

AAV5 Capsid

Two studies of the AAV5 capsid in humans were identified.

D'Avola et al. (2016) studied the AAV5 vector comprising the capsid shell of AMT-060 and containing a functional porphobilinogen deaminase (PBGD) gene tested at varying doses (5×10^{11} to 1.8×10^{13} gc/kg; single IV infusion) in eight adult subjects with intermittent acute porphyria. One subject failed screening due to pre-existing AAV5 neutralizing antibodies. All eight subjects who received AAV5-PBGD were followed for one year. No treatment-related adverse events (AEs) were observed during the infusion or the follow-up period. One subject who received the highest dose experienced a mild elevation (<3x upper limit of normal [ULN]) of liver transaminase levels one week after receiving AAV5-PBGD, coincident with an acute porphyria attack. Levels normalized once the attack resolved. As expected, all subjects developed antibody titers against AAV5; no subjects developed antibodies against the transgene product. The AAV5 vector was cleared from all bodily secretions by 30 days post-administration. Together, these findings support good safety and tolerability for the AAV5 capsid. Evaluation of liver biopsies at one year post-treatment showed persistence of vector genomes and transgene messenger ribonucleic acid (RNA) in all six subjects evaluated, suggesting that the AAV5 capsid is able to effectively deliver genetic material to the nuclei of liver cells.

Preliminary results up to 34 weeks post-treatment have also reported by Pasi et al. (2016) from a Phase 1/2 trial of an AAV5-B-domain-deleted factor VIII (FVIII) construct in 9 adults with hemophilia A. In this trial, subjects received doses ranging from 6×10^{11} to 6×10^{13} gc/kg via single IV infusion. No serious adverse events (SAEs) were observed. The first subject receiving the highest dose experienced a mild increase in transaminase levels, which resolved with a short course of corticosteroids; subsequent subjects received prophylactic steroid treatment, of whom three experienced mild transient increases in their transaminase levels. All subjects demonstrated increased FVIII activity with no decreases during these events.

Together, these findings support a strong expected safety profile of the AAV5 capsid in humans, with no serious safety concerns emerging. Mild, asymptomatic liver transaminase elevations are the most common event, consistent with reports from other liver-targeted AAV serotypes. Together, these studies provide evidence that the AAV5 capsid is capable of transducing human hepatocytes in vivo, resulting in detectable, sustained transgene expression.

Gene Cassette

Gene transfer of the human wild-type factor IX gene has been evaluated in three key studies in adult subjects with hemophilia B. The most recent reports demonstrate that this construct is capable of inducing sustained, clinically relevant increases in factor IX activity without associated safety concerns.

In three subjects receiving transfer of the wild-type human factor IX gene under control of a cytomegalovirus (CMV) promotor/enhancer (2×10^{11} gc/kg) into the muscle via AAV2, evidence of factor IX protein expression was seen both in the circulation and through histological examination of muscle biopsies (Kay et al., 2000). Factor IX consumption was reduced in two subjects, providing evidence of biological activity of the secreted protein. Five additional subjects were then dosed up to 1.8×10^{12} gc/kg. Presence and expression of the transgene was demonstrated via polymerase chain reaction (PCR) and Southern blot in muscle biopsies up to month 10 post-injection. No muscle tissue abnormalities were noted, suggesting the cells were not damaged by incorporation of the vector genome (Manno et al., 2003). Follow-up of one subject 3.7 years after injection showed persistence of the vector genome and protein expression locally in the muscle (Jiang et al., 2006).

A subsequent trial examined the effects of the wild-type human factor IX under control of a liver-specific human α1-antitrypsin (hAAT) promotor coupled with the apolipoprotein E (APOE) enhancer and hepatic control region, packaged into AAV2 vectors and administered via hepatic vein infusion to seven subjects at doses up to 2×10^{12} gc/kg (Manno et al., 2006). No evidence of increased circulating factor IX activity was obtained at 8×10^{10} or 4×10^{11} gc/kg. At the highest dose, both subjects showed transient increases in circulating factor IX activity (approximately 12% and 3% of normal) which gradually returned to baseline. The high-responding subject demonstrated asymptomatic increases in serum transaminase levels coincident with the decline in factor IX activity that subsequently normalized. A second subject who received the middle dose also experienced an asymptomatic elevation of serum transaminase levels that normalized without intervention. Testing in this subject indicated that no T-cell response developed to the factor IX protein.

The cassette identical to that incorporated in AMT-060, and forming the basis for the modified version, AMT-061, has been examined in 10 subjects in a trial sponsored by St Jude's and University College of London (Nathwani et al., 2011; Nathwani et al., 2014). Subjects received increasing doses (2×10^{11} to 2×10^{12} gc/kg; single IV infusion) of the LP1-hFIXco construct

delivered via AAV8, resulting in a steady state of 1-6% normal factor IX activity that remained stable at last reported follow-up (1.5-4.3 years after administration). Use of exogenous factor IX replacement was reduced by 92% following treatment. Bleeding episodes in the year after gene transfer decreased to a median of 1.5, compared with 15.5 in the year preceding treatment. Four of the six subjects who received the highest dose experienced increases in serum transaminase levels 7-10 weeks after administration and were treated with prednisolone, of whom three experienced an associated loss of factor IX activity. No subjects developed inhibitors to factor IX protein. All subjects demonstrated evidence of endogenous synthesis of factor IX from the transgene cassette. No evidence of late toxicity has emerged even at approximately 4.5 years post treatment.

Recently, preliminary results have been presented from a single study showing sustained factor IX expression driven by a gene cassette encoding a Padua variant FIX (George et al., 2017). Ten subjects received a single IV dose of 5 x 10^{11} gc/kg, resulting in mean steady state factor IX expression of approximately 29% in those followed for ≥ 12 weeks at time of the report. Two subjects experienced elevations of liver transaminases and were treated with prednisolone, of whom one experienced a partial loss of factor IX activity. No subjects developed inhibitors.

Across all three studies, no subjects who had received gene transfer (total N=25) developed inhibitors to the transgenic human factor IX protein (Manno et al., 2003; Manno et al., 2006; Nathwani et al., 2014; George et al., 2017).

1.3.2.2 Results of Clinical Trial-AMT-060-01

Enrollment and dosing in a first-in-human trial with AMT-060 (CT-AMT-060-01) has been completed. Seventy-eight weeks follow-up from all five subjects in the low dose (5 x 10^{12} cg/kg) cohort and 52 weeks follow-up from all five subjects in the high dose (2 x 10^{13} cg/kg) cohort are currently available. The durability of factor IX protein expression encoded by the codon optimized human coagulation factor IX cDNA obtained at this point in time is robust and sustainable.

Adverse Events

A total of 10 subjects have experienced 84 AEs; five subjects with 46 AEs in the low dose cohort and five subjects with 38 AEs in the high dose cohort. There have been no deaths and no AEs that have resulted in discontinuation from the trial. The majority of AEs were mild in severity/intensity (85%); only one AE has been categorized as severe, a myelopathy resulting in lower back pain in subject PPD. The majority of treatment emergent AEs (TEAEs) (70 of 84) were considered to be unlikely related to trial treatment. There were a total of four SAEs reported. In the low dose cohort, there were two SAEs: one SAE of mild elevation of alanine aminotransferase (ALT; assessed as serious based on criteria of medical significance) and the second SAE of short febrile episode (assessed as serious due to extension of hospital stay for one

day). Both SAEs were assessed as related to the drug product by the Investigator. In the high dose cohort, there were also two SAEs: one SAE of mild elevation of ALT, assessed as related to the drug product (SAE criterion of medical significance) and one SAE of myelopathy following surgery (SAE criterion was hospitalization), which was assessed as unlikely related. The outcomes of all SAEs were considered as recovered/resolved.

The most frequently experienced AEs were in the System Organ Class (SOC) Musculoskeletal and Connective Tissue Disorders (20 events: 16 events in Cohort 1 and 4 events in Cohort 2), followed by SOC Infections and Infestations (16 events: 7 events in Cohort 1 and 9 events in Cohort 2), SOC Nervous system disorders (8 events: 3 events in Cohort 1 and 5 events in Cohort 2), SOC General Disorders (7 events: 4 events in Cohort 1 and 3 events in Cohort 2), SOC Injury, poisoning and procedural complications (6 events: 2 events in Cohort 1 and 4 events in Cohort 2), SOC Investigations (5 events: 2 events in Cohort 1 and 3 events in Cohort 2), SOC Psychiatric disorders (5 events: 3 events in Cohort 2 and 2 events in Cohort 2).

ALT Elevations

Three subjects (one subject in the low dose cohort and two subjects in the high dose cohort) had mild, asymptomatic elevation of ALT and received tapering courses of prednisolone. This is a not an unexpected finding, as elevations of transaminases have been observed in clinical trials of gene therapy targeting the liver. It is important to note that in all three subjects none of the observed ALT elevations were associated with any concurrent loss of endogenous factor IX activity.

T-cell Activation

There were no sustained T-cell responses to the AAV5 capsid observed in either cohort. One subject in the low dose cohort had an isolated, low positive ELISpot measurement at Week 9 that was not associated with any clinical symptoms, elevation in liver enzymes, or change in factor IX activity. The general lack of ELISpot levels above threshold with no repeat elevations in any subjects and no clinical findings suggests that this one measurement is not clinically relevant.

Factor IX inhibitors

No subjects developed inhibitory antibodies to factor IX during the course of the trial.

Endogenous Factor IX Activity Levels

Following AMT-060, the mean of all endogenous factor IX activity values measured in the low dose cohort (Cohort 1) was 4.6% of normal (range of subject means: 1.3 - 6.8%), and remained stable during 78 to 91 weeks of follow-up. In the high dose cohort (Cohort 2), the mean of all factor IX activity levels was 7.1% (range of subject means: 3.1 - 12.7%) during 52 weeks of follow-up.

Two of the five subjects in the low dose and four of the five subjects in the high dose cohort have a mean factor IX activity of >5% of normal after treatment with AMT-060, which shifted them from severe or moderately-severe to a mild hemophilia phenotype, where spontaneous bleeding is rare. Four subjects, three in Cohort 1 and one in Cohort 2, shifted from a severe to moderate phenotype, where spontaneous bleeding occurs only occasionally. One subject in Cohort 1 shifted from a severe to moderately-severe phenotype, with endogenous FIX levels ranging from 1.1 to 1.4%.

Annualized Factor IX Replacement Therapy

Eight of the nine subjects who had been on factor IX prophylaxis at the time of trial entry no longer require factor IX prophylaxis after treatment with AMT-060. One subject in the low dose cohort remained on factor IX prophylaxis with factor IX expression <2%. Following successful (N=4) or attempted (N=1) cessation of prophylaxis after AMT-060, annualized exogenous factor IX use was reduced by 50 to 100% in the low dose cohort. The annualized relative reduction in factor IX replacement use ranged from 66 to 100% for four subjects after cessation of prophylaxis in the high dose cohort. For the subject who used factor IX replacement on-demand before and during the trial an 135% increase in the annualized factor IX use was observed, which was however mainly due to use for surgery.

Annualized Bleeding Rates

The modest increases in endogenous factor IX activity, leading to discontinuation of factor IX prophylaxis and large reductions in exogenous factor IX use, are paired with 58% and 84% reductions of annualized spontaneous bleeding rate in the low and high dose cohorts, respectively. All bleedings were considered of mild/moderate severity. Rates of traumatic bleeding were low and largely unaffected (11% decrease in the low dose cohort and no change in the high dose cohort).

Summary of the Preliminary Results:

Safety:

- The single IV infusion of AMT-060 was safe and well-tolerated in both the low- and high-dose cohorts
- The following SAEs were reported
    - two elevations of ALT
    - one short febrile episode that prolonged hospital stay (<24 hours)
    - myelopathy that required hospitalization
- No subjects developed inhibitory antibodies against factor IX
- No sustained capsid-specific T-cell responses were observed across both cohorts

- Three out of 10 subjects (2 out of 5 subjects in the high dose cohort) experienced mild, asymptomatic elevations of ALT, which were treated with a tapering course of corticosteroids.
- ALT elevations were not associated with a detectable activation of capsid-specific T-cell response and not correlated with a loss of endogenous factor IX activity

Efficacy:

- Clinically relevant endogenous factor IX activity (≥3%) was established in 9 out of 10 subjects across both cohorts (5 out of 5 in the high dose cohort)
- Continuous, regular prophylaxis was discontinued in 8 out of 9 subjects previously dependent on prophylaxis (4 out of 4 in the high dose cohort)
- Factor IX expression levels remain stable and durable for up to 1.5 years in the low dose and up to 1 year in the high dose cohort without indications of loss of expression
- Shift to mild hemophilia B phenotype (>5%) was achieved in
    - Two out of five subjects in the low dose
    - Four out of five subjects in the high dose cohort
- A reduction/elimination in the use of factor IX replacement therapy and a lowering of the annualized bleeding rate was demonstrated in both cohorts

1.4 Rationale for the Trial

The current treatment options for hemophilia B have several limitations. Treatment with prophylactic regular IV injections of factor IX is not curative and very demanding due to the need for frequent IV infusions and concomitant risk for infection and thromboses related to the placement of indwelling catheters. Periodic or regular factor IX infusion results in peaks and troughs in plasma factor levels allowing for breakthrough bleeding episodes. Due to these factors, poor adherence to treatment is a concern and a major contributing factor to failure of prophylaxis, associated with increased risk of bleeding and subsequent joint damage, thereby adding to the all-cause mortality rate. Despite the exogenous factor replacement, subjects may still experience breakthrough bleedings. There is also a risk of developing neutralizing antibodies against the administered factor IX. The burden of the disease is high, both for the individual subject and their families, and for society. Due to (long-term) impairments in mobility and functional status, subjects may not be able to fully participate in social activities, such as sports, school or work. Living with hemophilia 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. The cost of severe hemophilia, including indirect costs, is estimated at EUR 199,541 per subject in Europe, ranging from EUR 129,365 to 319,024 (O'Hara et al., 2017). Hemophilia subjects are accredited with requiring 2-3 times the health care resources per inhabitant in developed countries (Schramm & Berger, 2003). Hemophilia B thereby directly impacts the health-related CCI (Witkop et al., 2017).

Somatic gene therapy for hemophilia B offers the potential for shift of the disease severity from severe to a moderate or mild hemophilic phenotype or complete amelioration through continuous endogenous production of factor IX protein after a single administration of AAV vector particles. Even a small rise in constantly circulating factor IX protein can substantially ameliorate the bleeding phenotype.

Robust preliminary efficacy and safety results have been obtained with AMT-060 in an ongoing Phase 1/2 trial (CT-AMT-060-01) in 10 subjects with hemophilia B. No screening failures due to high titers of pre-existing neutralizing antibodies against AAV5 were encountered. An increased level of factor IX activity was observed after subjects in the low dose cohort and high dose cohort were treated with the AMT-060 at 5 x 10^{12} gc/kg and 2 x 10^{13} gc/kg, respectively. Clinically relevant endogenous factor IX activity (≥2%) was established in nine out of 10 subjects across both cohorts (five out of five in the high dose cohort), with two out of five and four out of five subjects achieving a shift to mild phenotype (>5%) in the low and high dose cohorts, respectively. Continuous, regular prophylaxis was discontinued in eight out of nine subjects previously dependent on prophylaxis (four out of four in the high dose cohort). As of writing, factor IX expression levels remain stable and durable for 1.5 years without indications of loss of expression. A reduction/elimination in the use of factor IX replacement therapy and a lowering in the risk of bleeding events over time were demonstrated in both cohorts. The single IV infusion of AMT-060 was safe and well-tolerated in both the low- and high-dose cohorts. The most frequent AE related to gene therapy targeting the liver is ALT elevation. One subject in the lower dose cohort and two subjects in the higher dose cohort had mild, asymptomatic, transient elevations of ALT. No change in factor IX activity and no T-cell response were seen in the subjects with ALT elevations. No subjects developed inhibitory antibodies against factor IX.

The clinical benefit of AMT-060 was achieved with a factor IX protein-to-activity ratio of approximately 1. Modifying AMT-060 with the implementation of the naturally occurring Padua variant is expected to result in a factor IX protein-to-activity ratio of approximately six. Although AMT-061 has yet to be studied in humans this design modification is anticipated to lead to a higher level of circulating factor IX activity at comparable levels of factor IX protein and thereby increase the likelihood of alleviating the need for exogenous therapy, including the need for on-demand treatment of traumatic bleeds and selective prevention of a bleed (e.g., because of upcoming physical activity, sports, etc.).

This isolated modification is a prospectively defined product improvement that will not influence other established safety characteristics of AAV5 at 2 x 10^{13} gc/kg such as the level of factor IX protein expressed with 2 x 10^{13} gc/kg AMT-061, immune response against AAV5, the occurrence of manageable ALT/AST (aspartate aminotransferase) elevations or risk of T-cell responses against transduced cells with potential concurrent loss of efficacy.

In addition to the above, the continuous accumulation of clinical data from the use of AAV5 in hemophilia subjects suggests that AAV5 is associated with a very low risk of inducing immune

responses that may lead to loss of the newly induced endogenous factor IX protein expression. Furthermore, low titers of pre-existing antibodies against AAV5 (using a highly sensitive assay), which may demonstrate neutralization of AAV5 transduction in in-vitro assays, have no apparent inhibitory effect in human studies at currently administered dose levels. As a consequence, future trials with AMT-061 (including this trial) will allow enrolment of subjects even in the presence of anti-AAV5 antibodies.

The efficacy (protein expression) and safety results obtained during the Phase 1/2 trial with AMT-060 demonstrate 2 x 10^{13} gc/kg to be the optimal dose for use in future trials in terms of safety and efficacy. In addition, accumulated clinical and non-clinical data support the implementation of the prospectively defined product enhancement of AMT-061 at 2 x 10^{13} gc/kg for the pivotal trial. The primary aim of this trial is to confirm that a single dose of 2 x 10^{13} gc/kg AMT-061 will result in factor IX activity levels of ≥5%. In addition, the safety profile of AMT-061 will be demonstrated.

1.5 Risk/Benefit Considerations

Somatic gene therapy for hemophilia B offers the potential benefit of a shift of the disease severity from severe to a mild hemophilia phenotype or complete amelioration through continuous endogenous production of factor IX after a single administration of vector.

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

The risks as described in the following sections have to be considered:

1.5.1 Risk of Infusion-related Toxicity

To date no infusion-related toxicities have been observed in previous clinical trials of liver-directed gene transfer, including the CT-AMT-060-01 trial. Nonetheless, as a precaution subjects in this trial will be monitored for tolerance to the IMP and detection of potential immediate AEs at the clinical trial site for 24 hours following infusion.

1.5.2 Risk of Immune Mediated Neutralization of the AAV5 Gene Therapeutic Vector

Pre-existence of antibodies that recognize a gene therapeutic vector can potentially reduce its bioavailability and hence its activity. For this reason, the eligibility criteria for CT-AMT-060-01 included the absence of detectable levels of circulating AAV5-neutralizing antibodies. Screening for AAV5 neutralizing antibodies was performed using a bio-assay based on a green fluorescent protein (GFP) reporter gene. In line with previously reported low prevalence for AAV5 neutralizing antibodies (Boutin et al., 2010), no antibodies were detected and all subjects passed screening.

Recent availability of a more sensitive bio-assay, based on a luciferase reporter gene prompted retrospective analysis of the screening samples from the CT-AMT-060-01 trial and pre-treatment samples of some of the nonclinical NHP studies. Retrospective analysis using this more sensitive assay revealed the presence of detectable levels of AAV5 neutralizing antibodies in three out of 10 CT-AMT-060-01 trial subjects and in all tested animals. However, judging by circulating factor IX activity levels or safety outcomes, these pre-existing AAV5 neutralizing antibody levels held no predictive value: the CT-AMT-060-01 trial subject showing the highest titer of 1/340 also showed highest circulating factor IX activity levels of this cohort, and in the NHPs no impact on efficacy was evident at titers up to 1/1000, at dose levels down to one-tenth of the low dose level in the CT-AMT-060-01 trial (i.e., 5×10^{11} gc/kg versus 5×10^{12} gc/kg).

These studies suggested that although AAV5 neutralizing antibodies are prevalent in humans and NHPs, the absolute levels at which they are present may not suffice to impact AMT-060 at the infused dose. In all subjects who were shown to have AAV5 neutralizing antibodies prior to treatment, factor IX activity remained stable, and none of the subjects showed evidence of liver toxicity or activation of T-cell responses against the capsid. As pre-existing antibody levels did not seem to preclude efficacy, and were not associated with any immune-mediated adverse effect, the proposed trial will allow enrolment of subjects regardless of antibody levels. Nonetheless, AAV5 neutralizing antibody levels will be measured in all subjects both before and after dosing, in order to allow a retrospective analysis to confirm the suggested lack of impact of prevalent titers on the efficacy of AMT-061. For this, the more sensitive luciferase-based assay will be used.

It is of note that after dosing of AMT-061 all subjects will develop antibodies against the capsid proteins and these antibodies are likely to persist. As liver transduction will have taken place before these responses are fully mounted, these responses are unlikely to impact factor IX expression. However, they may impact the efficacy of any future administration (i.e., a second dose) of the same vector, since post-dosing antibody levels may exceed prevalent pre-existing levels by several orders of magnitude.

1.5.3 Risk of Immune Mediated Liver Toxicity

Intravenous administration of a liver-directed AAV vector might lead to transaminase increase. Previous clinical trials have shown that increases in liver enzymes respond promptly and normalize after administration of glucocorticoids. Subjects in the trial will be monitored weekly during the first 12 weeks after infusion of AMT-061 for the occurrence of transaminase increases, which, in the absence of an alternative etiology for the ALT increase, may warrant the initiation of a corticosteroid treatment (see Section 5.6.4).

In the CT-AMT-060-01 trial, three subjects (one subject in the low dose cohort and two subjects in the high dose cohort) had mild, asymptomatic increase of ALT. This is not an unexpected finding, as increases of transaminases have been observed in trials associated with gene therapy

targeting the liver. It is important to note that in all three subjects none of the observed ALT increases was associated with any concurrent loss of endogenous factor IX activity. No evidence of immune reaction (e.g., capsid-specific T-cell response) was associated with this increase.

1.5.4 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 blood, urine, saliva, nasal secretion, faeces and semen. In trial CT-AMT-060-01, vector DNA was detected at 78 weeks and later in semen and blood in some subjects.

Vector DNA measured in these bodily fluids is unlikely to represent infectious particles. In addition, 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.

1.5.5 Risk of Vector DNA Integration into the Host Genome

Reaction (nr LAM PCR) and subsequent high throughput sequencing on DNA was extracted from the livers of both mouse and Cynomolgus macaques after administration of AMT-060 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 AMT-060 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.

1.5.6 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.

Additionally, subjects are asked to inform their partner of their participation in this trial, as well as the importance of contraception use to limit the reproductive potential in the period from IMP administration until AAV5 has been cleared from the semen.

1.5.7 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.

1.5.8 Risk of Inhibitor Formation to Protein Expressed from the Transgene

There is a risk of inhibitor/antibodies development against the expressed factor IX protein. No factor IX inhibitor formation was seen in any of the previous clinical trials reported in literature where subjects were exposed to human factor IX gene transfer and where the expressed levels of factor IX were measurable (Manno et al., 2003; Manno et al., 2006; Nathwani et al., 2011; Nathwani et al., 2014) or in CT-AMT-060-01. In-silico studies indicate no higher risk of potential immunogenicity for factor IX protein expressed from AMT-061 following incorporation of the single amino acid change as compared to the "wild-type" factor IX protein expressed from AMT-060. Expression of a Padua transgene product did not result in inhibitors in studies conducted in murine and inhibitor-prone canine models (Cantore et al 2012; Monahan, 2014; Crudele et al 2015).

To assist with minimizing this risk, subjects will be selected on the basis of a low risk of factor IX inhibitor development by choosing subjects with more than 20 exposure days to a factor IX product as well as omitting subjects with a previous factor IX inhibitor. Subjects will be regularly monitored for factor IX inhibitor development.

1.5.9 Risk of Breakthrough Bleeding

The scope of the liver-directed AAV gene therapy approach is to establish a stable and durable expression of factor IX and to convert to mild or completely ameliorate the severe hemophilia phenotype. Previous clinical trials with similar AAV vectors and the wild-type gene cassette have shown that stable and years long factor IX expression can be achieved (Nathwani et al., 2014). Nonetheless, there is a risk that breakthrough bleeding may occur, particularly if demanding physical activity is undertaken. This risk will be managed by the use of factor IX replacement as needed throughout the trial. Every effort will be made to avoid routine use of prophylactic factor IX replacement therapy during the trial.

In a previous trial with AMT-060, therapeutic levels of factor IX protein and resultant factor IX activity levels were seen as early as one week after treatment.

At baseline, subjects will be administered a challenge dose of short-acting factor IX (40 IU/kg) for the factor IX recovery assessment. This challenge dose should provide subjects sufficient factor IX coverage for the initial two to three days post treatment. Three days after IMP administration, the subjects should visit the clinic for endogenous factor IX activity assessment (by local laboratory). If the factor IX activity result is ≥5%, further factor IX prophylaxis will not

be given. If the factor IX activity result is <5%, another dose of short-acting factor IX will be administered to the subject and endogenous factor IX activity assessment will be performed again at the week 1 routine visit.

Additional on-demand factor IX may be given after treatment with AMT-061, if considered necessary. Furthermore, subjects are monitored closely for five years after administration of IMP and the need for renewed prophylactic therapy will be clinically assessed by the treating physician according to the local standard of care for hemophilia subjects.

1.6 Accommodations Due to the COVID-19 Pandemic

In the first quarter of 2020, a pandemic was announced for Coronavirus Disease 2019 (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 3.1.1 for details on measures related to adjustments to visit location/method and schedule, as well as Section 6.2.2.4 and Section 6.2.3.4 for details on the new visit windows for CCI questionnaires and abdominal ultrasound assessments, respectively. 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 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, it is mandatory to immediately notify appropriate authorities as per site's regulations and to notify Medpace and uniQure's Medical Directors. As co-infections can occur, all subjects should be considered 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.

2 TRIAL OBJECTIVES

2.1 Primary Objective

To confirm that a single dose of 2 x 10^{13} gc/kg AMT-061 will result in factor IX activity levels of ≥5% at six weeks after dosing

2.2 Secondary and CCI

Secondary Efficacy Objectives are focused on investigating the effect of 2 x 10^{13} gc/kg AMT-061 on endogenous factor IX activity at 52 weeks, discontinuation of previous continuous prophylaxis, total usage of factor IX replacement therapy, the annualized bleeding rate, and specific types of bleeding events (e.g., spontaneous bleeds, joint bleeds, traumatic bleeds). Specifically, the secondary objectives are the following:

- To assess effect of 2 x 10^{13} gc/kg AMT-061 on endogenous factor IX activity at 52 weeks
- To assess effect of 2 x 10^{13} gc/kg AMT-061 on discontinuation of previous continuous prophylaxis
- To assess effect of 2 x 10^{13} gc/kg AMT-061 on total usage of factor IX replacement therapy
- To assess effect of 2 x 10^{13} gc/kg AMT-061 on the annualized bleeding rate
- To assess effect of 2 x 10^{13} gc/kg AMT-061 on specific types of bleeding events (e.g., spontaneous bleeds, joint bleeds, and traumatic bleeds)

CCI

2.3 Safety Objectives

The Safety Objectives include monitoring of AEs, formation of anti-AAV5 antibodies (total immunoglobulin M and immunoglobulin G [IgM and IgG], neutralizing antibodies), AAV5 capsid-specific T cell response, formation of anti-factor IX antibodies, formation of factor IX inhibitors, hematology and serum chemistry, shedding of vector DNA in blood and semen, inflammatory markers, AST/ALT elevations, use of corticosteroids required to preserve factor IX activity in the context of AST/ALT elevations, abdominal ultrasound, and alpha-fetoprotein (AFP).

3 TRIAL DESIGN

3.1 Overall Trial Design

This is a Phase IIb, open-label, single-dose, single-arm, multi-center trial consisting of a screening, a treatment plus post-treatment follow-up, and a long-term follow-up phase.

After a screening period of maximal six weeks, eligible subjects will receive a single IV dose of 2×10^{13} gc/kg AMT-061 and will thereafter be followed for a total of five years (60 months). Post-treatment follow-up visits are planned as follows:

- Weekly up to Week 12
- Every 2nd week from Week 12 to Week 26
- Every month from Week 26 to Week 52

All subjects will continue to be followed every half year from Week 52 to 60 months (4 years long-term follow-up).

The subjects will be monitored for tolerance to the IMP and detection of immediate AEs for 24 hours (overnight stay) after dosing. The dosing of the subjects must be separated by a minimum of 14 calendar days to allow for subject safety monitoring and to ensure appropriate action can be taken in case any acute reactions are observed.

One interim analysis will be performed after six weeks post-dose to determine the efficacy of AMT-061 in terms of factor IX activity.

After 52 weeks post-dose and 2.5 years post-dose, efficacy and safety data will be collected on all subjects. These data will be locked, analyzed, and reported in a CSR (52-week analysis) and CSR addendum (2.5-year analysis). Data from that point will be considered locked and will not be changed. At the end of the 4-year long-term follow-up period (60 months/5 years post-dose), all safety and efficacy data will be reported in a CSR addendum covering the entire study period including the final 2.5 years post-dose follow-up period (5-year analysis).

The end of the clinical trial is defined as the point in time when the last subject has completed the overall follow-up observation period of 5 years after administration of the IMP.

3.1.1 Considerations Due to the COVID-19 Pandemic

Due to the COVID-19 pandemic, adjustments to the visit location/method or schedule may be made during the long-term follow-up to accommodate safety concerns and restrictions experienced by individual subjects and sites. In all cases, subjects will be kept informed, via 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-061 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 Sponsor). 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 site 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 factor IX consumption and confirm their use of the paper diaries (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.

3.2 Trial Design Rationale

In gene therapy it is common practice to provide a single dose of vector via IV infusion. This infusion in a previous trial with AMT-060 was proven to be safe and effective resulting in identification of a clincial dose for AMT-060 to proceed to further clinical studies. Although this resulted in successful conversion of clinical phenotype to mild in most subjects, the desired goal is to achieve higher levels of factor IX activity to allow for a more consistent and meaningful clinical response. This trial will investigate AMT-061 at the identified dose for AMT-060 (2×10^{13} gc/kg).

This is the first trial with the gene product AMT-061 in humans. As AMT-061 is an isolated modification of the wild-type human factor IX in AMT-060, it is expected that the safety of this product improvement will be similar as the safety characteristics that have been described for AMT-060 at a dose of 2×10^{13} gc/kg. Also, clinical benefit of AMT-060 was achieved at this dose (refer also to Section 1.4 for a description of the Phase 1/2 trial). Therefore, a similar dose of AMT-061 will be used in this trial. Based on NHP studies with AMT-061, as well as data from patients with the natural Padua mutation, it is expected that the modified product AMT-061 will lead to a higher level of circulating factor IX activity at comparable levels of factor IX protein and thereby increase the likelihood of alleviating the need for exogenous therapy,

including the need for on-demand treatment of traumatic bleeds and selective prevention of a bleed (e.g., because of upcoming physical activity, sports, etc.). At 2 x 10^{13}gc/kg, AMT-061 is expected to result in mean factor IX activity ranges of 40% of normal, ranging between approximately 18-76%.

This trial is conducted at multiple centers as the prevalence of severe and moderately severe hemophilia B subjects is low.

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.

3.3 Trial Endpoints

3.3.1 Primary Endpoint

The primary aim of the trial is to demonstrate that AMT-061 will provide a minimal therapeutic response of ≥5% factor IX activity using a dose of 2 x 10^{13} gc/kg. This is the proposed dose to be used in a further pivotal Phase 3 trial. The primary efficacy parameter is factor IX activity level at six weeks after dosing (at Week 6 post AMT-061 dose).

3.3.2 Secondary Endpoints

Secondary efficacy endpoints:

- Endogenous factor IX activity at Week 6 and Week 52 post AMT-061 dosing
- Remaining free of previous continuous prophylaxis during 52 weeks following AMT-061 dosing
- Total usage of factor IX replacement therapy until 52 weeks following AMT-061 dosing, excluding ad hoc prophylaxis for invasive procedures
- Annualized bleeding rate after 52 weeks of AMT-061 dosing (including a further break down of the frequency and percentage of spontaneous, traumatic, and joint bleeding events)

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Secondary safety endpoints

- AEs
- Hematology and serum chemistry parameters
- ALT/AST levels and corticosteroid use for ALT/AST elevations
- Parameters on antibody formation to AAV5 and human factor IX

- AAV5 capsid-specific T cell response
- Inflammatory markers
- Vector DNA in semen and blood
- AFP

Safety endpoints are observed over the 52-week post-treatment follow-up phase and for an additional four years in the long-term follow-up phase. An additional endpoint in the long-term follow-up is abnormal findings on the abdominal ultrasound.

3.4 Sample Size

A minimum of three subjects will be enrolled in the trial (see also Section 9.11). To account for drop-outs additional subjects may be recruited.

3.5 Sites and Regions

It is planned to conduct this trial in approximately three sites in the United States (US) and the European Union (EU).

4 TRIAL POPULATION

Subjects will be adult males with severe or moderately severe hemophilia B.

4.1 Inclusion Criteria

The subject cannot be enrolled in the trial before all of the following inclusion criteria (including test results) are met:

1. Male
2. Age ≥18 years
3. Subjects with congenital hemophilia B classified as one of the following:
   a. Known severe factor IX deficiency (<1% of normal circulation factor IX) for which the subject is either on continuous routine factor IX prophylaxis* or using on-demand factor IX replacement therapy
   b. Known moderately severe factor IX deficiency (1-2% of normal circulating factor IX, inclusive) and a severe bleeding phenotype as defined by at least one of the following:
      i. On continuous routine factor IX prophylaxis* for a history of bleeding
      ii. On demand factor IX replacement therapy with a history of frequent bleeding (4 or more bleeding episodes in the last 12 months) or chronic hemophilic arthropathy (pain, joint destruction, and loss of range of motion) in one or more joints
4. >20 previous exposure days of treatment with factor IX 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 three consecutively collected semen samples (this criterion is applicable also for subjects who are surgically sterilized)
6. Able to provide informed consent following receipt of verbal and written information about the trial

* Continuous routine prophylaxis is defined as the intent of treating with an a priori defined frequency of infusions (e.g., twice weekly, once every two weeks, etc.) as documented in the medical records.

4.2 Exclusion Criteria

Subjects are excluded from the trial if any of the following exclusion criteria are met:

1. History of factor IX inhibitors
2. Positive factor IX inhibitor test at screening
3. Screening laboratory values:
   a. ALT >2 times ULN
   b. AST >2 times ULN

c. Total bilirubin >2 times ULN
d. Alkaline phosphatase (ALP) >2 times ULN
e. Creatinine >2 times ULN

4. Positive HIV serological test at screening, not controlled with anti-viral therapy as shown by CD4+ counts ≤200/µL or by a viral load of >200 copies/mL
5. 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 RNA (HCV RNA) positivity, respectively, at screening
6. History of hepatitis B or C exposure, currently controlled by antiviral therapy
7. Known coagulation disorder other than hemophilia B
8. Thrombocytopenia, defined as a platelet count below 50×10^9/L, at screening
9. Known severe infection or any other significant concurrent, uncontrolled medical condition including, but not limited to, renal, hepatic, cardiovascular, hematological, 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
10. Known significant medical condition that may significantly impact the intended transduction of the vector and/or expression and activity of the protein, such as disseminated intravascular coagulation, accelerated fibrinolysis, and profound liver fibrosis
11. Known history of an allergic reaction or anaphylaxis to factor IX products
12. Known uncontrolled allergic conditions or allergy/hypersensitivity to any component of the IMP excipients
13. Known medical condition that would require chronic administration of steroids
14. Previous gene therapy treatment
15. Receipt of an experimental agent within 60 days prior to screening
16. Current participation or anticipated participation within one year after IMP administration in this trial in any other interventional clinical trial involving drugs or devices

4.3 Reproductive Potential

Male subjects who participate in this trial accept 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 three consecutively collected semen samples (this criterion is applicable also for subjects who are surgically sterilized).

Subjects are being asked to inform their partner of their participation in this trial, as well as the importance of contraception use to limit the reproductive potential in the period from IMP administration until AAV5 has been cleared from the semen.

4.4 Restrictions

There is one restriction associated with participation in this trial, which is related to reproduction and described in Section 4.3.

4.5 Withdrawal of Subject from Therapy or Assessment

A subject may withdraw from the trial at any time, for any reason, without prejudice to their future medical care by his/her physician or at the institution. The Investigator or Sponsor may withdraw the subject at any time if it is not in the best interest of the subject to continue participation. Since this is a gene therapy trial in which the IMP is administered to human subjects as a one-time only dose, the Investigator should make all reasonable attempts to maintain the subjects in the trial after IMP administration to allow long-term follow-up on safety. Where a subject is withdrawn from the trial at his own request or based on a decision of the Investigator, the safety follow-up should be maintained, conditional to the consent of the subject. The safety follow-up will include periodic review (approximately every 6 months) of medical records to gather information obtained during routine visits from the subject with his treating physician for the time until 5 years post IMP administration. Information on AEs, SAEs, concomitant medication use, and laboratory assessments will be collected as available. If a subject is to withdraw from the trial, the Investigator should make all reasonable attempts to have the subject sign the separate informed consent form (ICF) in order to maintain the long-term safety follow-up.

Subjects withdrawing from the trial will be requested to complete the same final evaluations (see Section 4.1) as subjects completing the trial according to the protocol, particularly safety evaluations in the subject's interest so that data can be recorded in the same way as for subjects who completed the trial. Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for (if given) and date of withdrawal, must be recorded on the electronic case report form (eCRF) and source documents.

Subjects who withdraw prior to IMP administration or within the first six weeks after IMP administration will be replaced. Subjects who discontinue after six weeks post IMP administration will not be replaced.

4.5.1 Reasons for Discontinuation

The reasons for discontinuation from this gene therapy trial include:

- AE
- Withdrawal by principal Investigator
- Withdrawal by subject
- Lost to follow-up

4.5.2 Subjects 'Lost to Follow-up' Prior to Last Scheduled Visit

At least three documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact (office visit or telephone contact). One of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return the e-diary device (if applicable) and return to the site for final safety evaluations.

5 TRIAL TREATMENT

5.1 Treatment(s) Administered

The IMP is identified as AAV5-hFIXco-Padua (hereafter referred as AMT-061; etranacogene dezaparvovec). AMT-061 is a recombinant adeno-associated viral vector of serotype 5 (AAV5) containing the Padua variant of a codon-optimized human factor IX cDNA under the control of a liver-specific promoter. The pharmaceutical form of AMT-061 is a solution for IV infusion.

Subjects will receive a single IV infusion of 2 x 10^{13} gc/kg AMT-061.

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5.3 Dosing and Administration

AMT-061 will be administered at a dose of 2 × 10^{13} gc/kg as a one-time infusion in a peripheral vein. The subjects will be monitored for tolerance to the IMP and detection of immediate AEs for 24 hours (overnight stay) after dosing.

The dosing of the subjects must be separated by a minimum of 14 calendar days to allow for subject safety monitoring and to ensure appropriate action can be taken in case any acute reactions are observed.

Detailed instructions for IMP handling e.g., receipt, storage, preparation, administration, cleaning, destruction and the recording of these critical activities will be described in the IMP Handling Manual.

5.4 Randomization and Blinding

Not applicable, as this is an open-label trial with one treatment arm.

5.5 Labeling, Packaging, Storage, and Handling

All medication used in this trial will be prepared and labeled according to the rules of Good Manufacturing Practice, International Council for Harmonisation (ICH)-Good Clinical Practice (GCP) (E6[R2]) and local regulatory requirements. Further details on IMP labeling, packaging and handling, e.g., receipt, storage, preparation, administration, cleaning, destruction, documentation etc., will be described in the IMP Handling Manual.

5.6 Prior and Concomitant Medication/Therapy

5.6.1 Prior Medication/Therapy

Prior medication/therapy includes medication/therapy (including herbal treatments, vitamins, non-pharmacological treatment such as psychotherapy as appropriate) received within 30 days of and discontinued prior to the date of screening. Prior medication/therapy information must be recorded on the appropriate eCRF page.

For this trial, it is not allowed to have received an experimental agent within 60 days prior to screening. In addition, use of previous gene therapy is not allowed.

5.6.2 Concomitant Medication/Therapy

Concomitant medication/therapy is defined as any medication/therapy being continued by the subject at the date of screening, and any new medication/therapy received during the trial. Concomitant medication/therapy must be recorded on the appropriate eCRF page.

The following treatments will not be allowed during trial participation:

- Continuous routine factor IX prophylaxis post-dose if a subject's endogenous factor IX activity result is above 5%
- Treatment in another interventional clinical trial involving drugs or devices within one year from IMP administration in this trial
- Another gene therapy treatment
- Chronic administration of steroids (oral and/or inhaled)

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

At baseline, subjects will be administered a challenge dose of short-acting factor IX (40 IU/kg) for the factor IX recovery assessment. This challenge dose should provide subjects sufficient factor IX coverage for the initial two to three days post treatment. Three days after IMP administration, the subjects should visit the clinic for endogenous factor IX activity assessment. If the factor IX activity result is ≥5%, further factor IX prophylaxis will not be given. If the factor IX activity result is <5%, another dose of short-acting factor IX will be administered to the subject and endogenous factor IX activity assessment will be performed again at the Week 1 routine visit.

Additional on-demand factor IX may be given after treatment with AMT-061, if considered necessary. If the endogenous factor IX activity result is ≥5%, management will be based on the Investigator's clinical judgement and subject preference. Re-initiation of factor IX continuous routine prophylaxis may be considered if the endogenous factor IX activity is between 2 and 5% in at least two consecutive laboratory measurements, based on the Investigator's clinical judgment and subject preference. If the endogenous factor IX activity is below 2% in at least two consecutive central lab measurements, continuous routine factor IX prophylaxis may be restarted.

Factor IX infusions are not recommended for subjects with factor IX activity in the non-hemophilic (≥40% of normal) range especially in subjects with a confirmed COVID-19 infection, as increased thrombogenic risk is a known complication of COVID-19. Subjects with factor IX activity in the non-hemophilic range post-treatment with AMT-061 and infected with COVID-19 may potentially be at the same risk of thrombosis as subjects without hemophilia; antithrombotic therapy for these subjects should be considered under the same guidelines recommended for those without hemophilia.

5.6.3 Guidelines for Use of Factor IX for Subjects Undergoing Major Surgery

Replacement of factor IX for subjects undergoing major surgery in this trial will be according to established guideline (Srivastava et al., 2013) in terms of factor IX activity level pre and post-surgical procedure. The target factor IX activity level pre and post-major surgery regardless of whether the regular or extended half-life replacement is administered as per World Federation of Hemophilia is as follows:

	Factor IX Activity Level
Pre-operative:	60-80%
Post-operative:	40-60% Day 1-3
	30-50% Day 4-6
	20-40% Day 7-14

For minor surgery, use of factor IX is up to the Investigator but should be discussed with the medical monitor.

Factor IX infusions are not recommended for subjects with factor IX activity in the non-hemophilic (≥40% of normal) range especially in subjects with a confirmed COVID-19 infection, as increased thrombogenic risk is a known complication of COVID-19. Subjects with factor IX activity in the non-hemophilic range post-treatment with AMT-061 and infected with COVID-19 may potentially be at the same risk of thrombosis as subjects without hemophilia; antithrombotic therapy for these subjects should be considered under the same guidelines recommended for those without hemophilia.

5.6.4 Guidelines for Transaminase Elevations

Transaminase levels will be monitored based on the site's local laboratory results and central laboratory results, with local laboratory analysis results arranged, if possible, to be provided on the same day or the day after blood sampling has occurred to allow for rapid detection of any elevations in transaminase levels.

For ALT level increments of at least 2-fold baseline (i.e., pre-IMP) and/or > ULN, by local or central laboratories, the Investigator should contact the Medpace and uniQure Medical Directors to discuss a clinical management plan on a case-by-case basis, including potential re-tests and/or initiation of corticosteroid treatment. In case of AST level increments > ULN, the Investigator should contact the Medpace and uniQure Medical Directors and a similar discussion should take place.

Investigators should assess potential causes of a transaminase elevation, to rule out if the elevation is due to intense exercise, alcohol consumption, or use of concomitant medications. Additional laboratory assessments including creatine kinase assessment and a viral panel are recommended as needed.

See Table 7 for a recommended approach to prednisolone treatment. Medications equivalent to prednisolone may also be used. A combined immunosuppressant regimen or the use of other products can also be considered in case of prednisolone treatment failure or contraindication. Corticosteroid tapering should be discussed among the Investigator and Medpace and uniQure Medical Directors based on changes in and normalization of transaminase levels.

Investigators should monitor subjects for corticosteroid-related AEs. If use of high dose prednisolone/prednisone is prolonged, blood pressure and glucose levels should be monitored at each clinic visit, or more frequently if needed, and Investigators should consider starting subjects on vitamin D, a proton pump inhibitor, and/or *Pneumocystis jiroveci* prophylaxis therapy.

Subjects who have been placed on corticosteroid treatment should be closely monitored for potential COVID-19 infection. Where possible and as per site guidelines and at the discretion of the Investigator, subjects should be tested for COVID-19 at the time of initiating corticosteroid treatment. Those on corticosteroid treatment who are positive for COVID-19 might be

considered for more rapid tapering than outlined in Table 7; corticosteroid tapering should be discussed among the Investigator and Medpace and uniQure Medical Directors.

Table 7 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 transaminase returns to baseline level (Day 0, pre IMP)	20
After pre IMP level has been reached	Reduce daily dose with 5 mg/week

5.7 Treatment Compliance and Drug Accountability

Investigators will be provided with a subject pack containing sufficient vials of the IMP to prepare and administer the required dose for each subject. The Investigator or designee will acknowledge receipt of the subject pack by documenting date of receipt, shipment content and condition. Accurate records of all IMP prepared, administered, returned, and/or destroyed must be maintained as detailed further in this section as well as in the IMP Handling Manual. Investigators will be responsible for implementing a system for subject and product traceability at the clinical site. That system should contain sufficient detail to allow linking of each vial delivered to the Investigator to the subject receiving it and *vice versa*.

The Investigator has overall responsibility for preparing and administering the IMP. Where permissible, tasks may be delegated to a qualified designee (e.g., a pharmacist) who is adequately trained in the protocol and procedures as described in the IMP Handling Manual and who works under the supervision of the Investigator. This delegation must be documented in the applicable trial delegation of authority form.

The Investigator or his/her designee (as documented by the Investigator in the applicable trial delegation of authority form) will administer the IMP only to subjects included in this trial, for whom it is confirmed that they are eligible for dosing, following the procedures set out in the trial protocol and the IMP Handling Manual. Each subject will be given only the IMP carrying his treatment assignment. All dispensing will be documented on the eCRFs and/or other IMP record.

The Sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records.

Based on entries in the site drug accountability forms, it must be possible to reconcile IMPs delivered with those used and destroyed if unused. All IMPs must be accounted for and all discrepancies must be investigated and documented to the Sponsor's satisfaction.

6 TRIAL SCHEDULE AND ASSESSMENTS

6.1 Trial Schedule

For details on the timing and frequency of the assessments and testing during Week -6 to 52 refer to the flow charts in Table 1 and Table 2, and for the period of 12 to 60 months refer to Table 3 and Table 4.

6.1.1 Screening (Week -6 to Day 0)

Informed consent must be obtained from each subject prior to any of the trial procedures are performed (see also Section 10.3.1).

Maximal six weeks prior to baseline, subjects are screened for eligibility, and historical bleeding and factor IX use data will be collected. Baseline is defined as Day 0 prior to IMP administration (Day 0 pre IMP). The screening period might be prolonged in consultation with the sponsor in case IMP administration needs to be delayed because of subjects experiencing a bleeding episode, surgery, or other event after screening which warrants this delay.

The electronic diary (e-diary) will be handed over to the subjects, and the Investigator/study nurse will train them in recording of the bleeding episodes and use of factor IX replacement therapy. From screening onwards, subjects will record their use of factor IX replacement therapy and bleeding episodes in the dedicated e-diary. e-Diary data will be reviewed on a continuous basis by the Investigator/study nurse. The period from screening up to baseline is considered a training period, after which the Investigator/study nurse will review and evaluate any problems with recording of e-diary data with the subject. The e-diary training can be repeated at any time during the trial as considered necessary by Investigator/study nurse.

Eligibility according to the trial in- and exclusion criteria will be evaluated at Screening and during the period up to baseline (i.e., Day 0 pre IMP). For a description of the in- and exclusion criteria please refer to Section 4.1 and Section 4.2.

The overall eligibility will be determined once all screening values and results of other required procedures are available. Subjects who fail to meet inclusion criteria and/or meet at least one of the exclusion criteria and did not receive AMT-061 are defined as a screen failure. Subjects qualifying as screen failures may be re-evaluated once for participation in the trial in consultation with the Sponsor.

Separate informed consents are to be taken for a blood sample for future research, and for factor IX gene sequencing analyses to be performed only if factor IX gene mutation information is not available. If the subject provides consent for one or both ICFs, the respective blood samples will be taken during the screening visit.

The use of concomitant medication and occurrence of AEs will be monitored throughout the trial on an ongoing basis.

6.1.2 IMP Administration

The day of IMP administration should be planned to take place as an overnight stay at the clinic and should take place within a maximum of six weeks after the Screening visit (unless sponsor approved a prolonged screening period as described in Section 6.1.1).

The baseline visit should also be planned as such that the baseline blood sample for factor IX activity and factor IX protein concentration assessment will be drawn at the point in time where factor IX activity and factor IX protein concentration is expected to be closest to trough levels (i.e., the baseline visit should be scheduled on days when routine factor IX prophylaxis is due to be administered). In case of on-demand factor IX replacement therapy use in proximity to the scheduled visit, the exogenous factor IX is to be washed out and the visit is to be rescheduled to the extent the visit window allows. The wash out period will be minimal 10 days for short-acting factor IX or more for long-acting factor IX (depending on half-life).

6.1.2.1 Baseline - Day 0 pre IMP

Subjects will be admitted to the hospital for an overnight stay.

It is recommended that each subject should complete the CCI [redacted], prior to the interview by the Investigator and/or study nurse and any other trial related procedures.

Prior to administration of AMT-061, the Investigator should ensure subject's eligibility (see Section 4.1 and Section 4.2). If a subject, who was previously considered eligible, no longer meets the trial inclusion criteria, the subject can be re-evaluated once, as described in Section 6.1.1.

The medical history will be evaluated, vital sign measurement, a physical examination and CCI [redacted] will be performed. Furthermore, baseline samples are taken for efficacy and safety laboratory parameters, serum chemistry and hematology. Also, for subjects who signed the optional ICF, the sample for future research is taken.

If not taken at screening, the optional blood sample for factor IX gene sequencing will be taken (if the subject has signed the separate ICF and only if information on factor IX gene mutation is not available).

From baseline until week 52, subjects continue with recording their use of factor IX replacement therapy and bleeding episodes in the e-diary. Diary data will be reviewed on a continuous basis by the Investigator/study nurse. In addition to the subjects' reporting of presumed bleeding episodes in the e-diary, the Investigator or designee will assess each bleeding episode, by

describing the circumstances as well as the nature of the reported bleed in a bleed specific narrative as soon as possible but at least within 72 hours after it has been reported by the subject. In case the information provided in the e-diary is not sufficient to assess the presumed bleeding and describe it in a narrative, the subject needs to be called and/or visit the site. e-Diary training should be repeated at any time during the trial as considered necessary by Investigator/study nurse.

6.1.2.2 IMP Administration and Post IMP

Subjects will receive a single infusion of AMT-061 according to the procedures described in Section 5.3 and in the IMP Handling Manual. After IMP administration (post IMP), 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).

After completion of the IMP infusion:

- Vital signs are measured at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours
- Blood samples are taken for vector genome detection at 1, 2, 3 and 24 hours
- A blood sample is taken for c-reactive protein (CRP) measurement at 3 hours

The subject may leave the clinic after all assessments at 24 hours after AMT-061 administration have been performed, and the subject received his subject treatment card.

6.1.3 Post-Treatment Follow-up

During the first year of follow-up, the recording of e-diary data by the subject will be continued as well as continuous review of these data, and adjudication of the reported bleeding episodes, by the Investigator/study nurse, as described in Section 6.1.2.1. In addition, use of concomitant medication and occurrence of AEs will be continuously monitored.

A physical examination and samples for efficacy and safety laboratory parameters, hematology and serum chemistry, are taken at each visit, except for the samples for factor IX antibodies and factor IX inhibitors (taken at Week 6, 12, 26 and 52), samples for Total and Neutralizing antibodies to AAV5 (taken at Week 1, 2, 3, 4, 5, 6, 12, 26 and 52), semen sampling for shedding (taken at Week 6, 12, 16, 26 and 52), and the blood sample taken for future research (taken at Week 12 and 52) (see also Table 2).

At Week 26 and 52, it is recommended that each subject should complete the CCI [redacted] prior to the interview by the Investigator and/or study nurse and any other trial related procedures.

The CCI [redacted] is evaluated at Week 52.

At six weeks after IMP administration, the factor IX activity data will be collected for interim analysis and reporting of this primary efficacy parameter.

At the Week 52 visit, the e-diary data devices are collected, and the paper diaries for the long-term follow-up are handed out. After the 52 week post-dose visit, the complete set of efficacy and safety data are collected. These data will be locked, analyzed, and reported in a CSR. Data from that point will be considered locked and will not be changed.

After the 2.5-year post-dose visit, the complete set of efficacy and safety data since the 52-week lock will be collected. These data will be locked, analyzed, and reported in a CSR addendum. Data from that point will be considered locked and will not be changed.

6.1.3.1 Final Visit or Early Discontinuation

The procedures listed for the final visit, with the exception of the distribution of the study-specific paper diaries, must also be performed at early discontinuation (refer also to Section 4.5). If a subject discontinues prior to Week 52 he must complete all Week 52 assessments/procedures. In case a subject discontinues after Week 52 but prior to Month 60 he must complete the Month 60 assessments/procedures.

6.1.4 Long-term Follow-up

Subjects will visit the clinic every 26 weeks (6 months ± 2 weeks). During these visits, it is recommended that the subject completes the CCI (only at Month 24, 36, 48, and 60), prior to any other trial procedure is performed. Further, a physical examination, evaluation of the CCI (only at Month 24, 36, 48, and 60), sampling for efficacy and safety laboratory parameters, and review of AEs and concomitant medications will be performed. An abdominal ultrasound will be performed at the Month 30, 36, 42, 48, 54, and 60 visits. In addition to AE review during these visits, there should be an additional contact moment between site staff and subject in between these routine visits to facilitate at least quarterly monitoring of occurrence of AEs. Options for how visits may occur to accommodate safety concerns and restrictions due to COVID-19 are described in Section 3.1.1.

In the long-term follow-up phase, subjects will document their use of factor IX replacement therapy and bleeding episodes in study-specific paper diaries. Subjects are expected to bring their long-term follow-up bleed diaries and long-term follow-up factor IX use diaries to every study visit during the long-term follow-up phase. At each visit, site staff will collect the information that is new since the previous visit in the paper diaries. In between study visits, subjects should contact the site staff immediately in case of an experienced bleed and/or factor IX use in addition to completing the questions/information requested on the paper diaries to capture all information.

In total, each subject will be followed for five years after administration of AMT-061. This is in line with the European Medicines Agency (EMA) guideline on follow-up of subjects administered with a gene therapy medicinal product (EMA/CHMP/GTWP/60436/2007) and the Food and Drug Administration (FDA) guidance on long-term follow-up after administration of human gene therapy products.

At the end of the long-term follow-up all end-of-trial procedures, i.e., the Month 60 procedures, as detailed in Table 3 and Table 4, should be taken. At the end of the trial, all safety and efficacy data will be reported in a CSR addendum (5-year analysis).

6.1.5 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 additional visit may include additional assessments, as deemed necessary by the Investigator, such as (but not limited to) physical examination, AE assessment, bleeding adjudication and/or repetition of instructions to the subject regarding subject e-diaries (Section 6.1.1), additional blood and/or semen sampling, repetition of blood sampling due to erroneous results (Section 6.2.4.2), or conduct of measurements that were missed at the previous visit.

6.1.6 Additional Care of Subjects after the Trial

No after care (i.e., after the long-term follow-up) is planned for this trial.

6.2 Trial Evaluations

6.2.1 Demographic and Other Baseline Characteristics

6.2.1.1 Demographics

Demographics collected at Screening include date of birth (i.e., age at screening visit), race, ethnic group and gender according to local regulations.

6.2.1.2 Medical History and Concomitant Illnesses

Medical history is any previous medical condition or surgical event, i.e., a condition/event that started prior to the screening visit, but is not ongoing at the screening visit. A concomitant illness is a medical condition that is ongoing at the screening visit.

Collection of historical medical information regarding hemophilia B is described in Section 6.2.1.4.

At Screening, 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 Screening 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 Screening if judged relevant by the Investigator (for example conditions that the Investigator evaluates could re-emerge over time, e.g., cancers).

6.2.1.3 Prior and Concomitant Medication/Therapy

For the definition of prior medication/therapy, refer to Section 5.6.1. For the definition of concomitant medication/therapy, refer to Section 5.6.2. 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 medication, vitamin and/or mineral supplements, homeopathic remedies and herbal preparations) used and changes in the use of medication. Refer to Section 6.2.2.1 for instructions on recording of factor IX replacement therapy. The Investigator should also record other concomitant treatments/therapy, e.g., physiotherapy.

The following information will be recorded on concomitant medication/therapy:

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

6.2.1.4 Hemophilia B Status and History

At Screening, the following medical history data related to hemophilia B will be recorded:

- Date of initial diagnosis
- Endogenous factor IX activity level at diagnosis (if available)
- Date and value of most recent endogenous factor IX activity assessment (if available)
- Severity of Hemophilia B
- Family members with a history of factor IX inhibitors
- Arthropathy
- CCI [redacted] as measured with CCI [redacted] version 2.1 (see Section 6.2.2.5)
- Number and location of target joints (defined as three or more spontaneous bleeds into a single joint within a consecutive six-month period. Where there have been ≤2 bleeds into the joint within a consecutive 12-month period the joint is no longer considered a target joint)
- Registered name and dosage regimen of current continuous routine prophylactic factor IX replacement therapy (if applicable)
- Hemophilia B related Surgical History
    - Date of surgery
    - Surgical event
    - Preventive treatment during surgery: recombinant or plasma product

6.2.1.5 History of Bleeding and Factor IX Use

At Screening the following historical information regarding bleeding and factor IX use will be collected and recorded in a specific module of the eCRF:

For the overall period prior to screening:

- Number of exposure days prior to trial entry. An exposure day is a day where the subject received one or more infusion(s) of factor IX replacement therapy. Additional information will be recorded for factor IX replacement therapy administered during the last 30 days prior to trial entry (trade name of factor IX medication, date & time of administration, units per administration).

Factor IX use data from one year prior to screening:

- Number of months on factor IX replacement therapy
- Dose and frequency of dosing; in case the subject is on intermittent prophylactic factor IX replacement therapy, each regimen followed in the last 52 weeks should be reported
- Recombinant or plasma factor IX product
- Number of treatment requiring bleeding episodes during each specific factor IX regimen
- Average number of units to treat a bleed during each specific factor IX regimen
- Number of bleeding episodes prior to initiation of prophylactic factor IX replacement therapy.

Bleeding data from one year prior to screening:

- Date of the bleed
- Type of bleed (spontaneous, traumatic, instrumented, unknown)
- If factor IX replacement therapy is provided, the name and total dose of the therapy

Information on invasive procedures requiring factor IX use in the year prior to screening:

- Date of procedure
- Type of procedure
- Factor IX product used (Registered drug name)
- Total factor IX dose (in IU) used for each procedure

6.2.1.6 Factor IX Gene Sequencing

Available information on factor IX gene mutation will be collected at Screening and recorded.

For those subjects who have given their consent, a blood sample for the purpose of factor IX gene sequencing analysis will be collected (preferably at Screening, but otherwise at a later time

point during the subject's trial participation), but only if information of factor IX gene mutation is not available and if separate informed consent is given by the subject.

Subjects who do not wish to participate in the factor IX 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 factor IX gene sequencing analysis.

Gene sequencing analysis will be performed at a central laboratory.

6.2.2 Efficacy Evaluations

For details on the timing and frequency of the assessments refer to the flow charts in Table 1 through Table 4.

The name and address of each laboratory used in this trial will be maintained in the Investigator's files at each site.

Details for the laboratory processing instruction will be provided in the laboratory manual.

6.2.2.1 Factor IX Replacement Therapy

From Screening until Week 52, subjects will be asked to record the all use of prophylactic and on-demand factor IX replacement therapy in an e-diary. The subject e-diary will include questions with respect to:

- Reason for factor IX use (i.e., continuous routine prophylaxis, selective prevention of a bleed [e.g., because of upcoming physical activity, sports, etc.], prophylaxis for invasive procedures, or other).
- Date and time of factor IX infusion
- Factor IX product used (Registered drug name)
- Total factor IX dose in International Units (IU)

The Investigator/study nurse will review the e-diary entries for completeness and accuracy against the subject's medical/hospital records.

Information on actual factor IX replacement therapy used will be recorded by the subject in the e-diary. In addition, the prescribed factor IX replacement therapy regimen will be recorded in the eCRF.

During the long-term follow-up phase, subjects will be expected to continue documenting factor IX replacement therapy in their study-specific paper long-term follow-up factor IX use diary, which they will bring with them to all study visits.

Factor IX infusions are not recommended for subjects with factor IX activity in the non-hemophilic (≥40% of normal) range especially in subjects with a confirmed COVID-19

infection, as increased thrombogenic risk is a known complication of COVID-19. Subjects with factor IX activity in the non-hemophilic range post-treatment with AMT-061 and infected with COVID-19 may potentially be at the same risk of thrombosis as subjects without hemophilia; antithrombotic therapy for these subjects should be considered under the same guidelines recommended for those without hemophilia.

6.2.2.2 Bleeding Episodes

From Screening until Week 52, subjects will record information of bleeding episodes in an e-diary. The subject e-diary will include questions regarding each bleeding episode with respect to:

- Date and time of onset of bleed (start and stop)
- Location of bleed
- Circumstances of bleed: spontaneous, traumatic, medical/dental/other procedure, unknown cause
- Location and type of bleed
- Symptoms associated with the bleed
- Tests performed
- Treatment of bleed with factor IX, and response to this treatment
- Treatment other than factor IX (e.g., compression, ice, pain medication, rest)

During the long-term follow-up phase, subjects will be expected to continue documenting bleeding episodes in their study-specific paper long-term follow-up bleed diary which they will bring with them to all study visits.

In case of the occurrence of a presumed bleeding as reported by the subject in the e-diary, the Investigator or designee needs to assess the bleeding as soon as possible but at least within 72 hours after it has been reported by the subject. In case the information entered in the e-diary is not sufficient to assess the bleeding, the subject needs to be called and/or visit the site.

The Investigator will assess the bleed according to local standard of care (including potential imaging). The bleeding data and outcome should be recorded in source documents.

Recurrent bleed: A bleed is defined as a recurrent bleed when, after no or minimal response to treatment, the bleed is occurring within 72 hours after stopping treatment for the original bleed for which treatment was initiated.

Persistent bleed: A bleed is defined as a persistent bleed when the same bleed continues for more than 72 hours in the same location, without stopping treatment for the original bleed for which treatment was initiated.

6.2.2.3 Factor IX Activity Levels and Factor IX Protein Concentration

Blood samples for determination of endogenous factor IX activity and factor IX protein will be collected and assessed at the central and/or local laboratory as indicated in the flow chart (Table 2). Central laboratory results for factor IX activity will be used in the analyses and local laboratory results for factor IX activity will be used for local monitoring of subjects.

Throughout the study, it will be the aim to draw blood samples at those time points when the subject's factor IX activity is expected to be at its trough.

For subjects on routine prophylactic factor IX replacement therapy:

- The Investigator and/or study nurse will collaborate with the subject to schedule study visits to take place on days when continuous routine prophylactic factor IX replacement treatment is due to be administered. At these visits, blood sampling will then take place just prior to administration of prophylactic factor IX replacement therapy at the clinic.
- If a subject uses additional on-demand factor IX replacement treatment, his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit takes place at the time the subject resumes his routine prophylaxis schedule.

For subjects only using on-demand factor IX replacement therapy (only applicable after AMT-061 administration):

- If a subject uses on-demand factor IX replacement treatment, his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit does not take place within 10 days of any on-demand factor IX product use.

Factor IX activity will be assessed by using the one-stage aPTT assay and a chromogenic assay (central laboratory). No additional blood will be drawn for chromogenic assays of factor IX activity; the test will be performed on back-up samples.

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6.2.3 Safety Evaluations

6.2.3.1 Adverse Events

All AEs will be collected from signing of the informed consent form until the end of the five-year follow-up.

At each trial visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (e.g., "Have you had any health problems since your last visit?"). During the long-term follow-up, AEs should be assessed at least quarterly by means of an additional contact moment between site staff and subject in between the scheduled visits. AEs are collected from the time informed consent is signed.

For definitions of (S)AEs, and procedures regarding reporting of (S)AEs refer to Section 7.

6.2.3.2 Vital Signs

Vital signs (blood pressure, pulse and body temperature) will be measured before and at specific time points after IMP administration on Day 0 (see Table 1). 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.

Abnormalities (e.g., high blood pressure) identified at the Screening will be documented in the subject's source documents and on the medical history eCRF. Changes after the Screening Visit will be captured as AEs on the AE eCRF page, as deemed clinically significant in the opinion of the Investigator. These abnormalities are to be followed until they reached "final outcome" (refer to Section 7.9).

6.2.3.3 Physical Examination (Including Height and Weight)

A physical examination will be performed at all visits, except for height and weight, which will only be measured at screening.

Height (without shoes) will be measured and recorded, rounded to the nearest centimeter. Body weight (without overcoat and shoes) will be measured and recorded, rounded to the nearest kilogram.

At Day 0, the physical examination will be performed prior to 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 need to be specified and recorded.

Abnormalities (e.g., scar at the left side at knee following total knee replacement, or arthropathy of left ankle due to hemophilia B) identified at the Screening will be documented in the subject's source documents and on the medical history eCRF. Changes after the Screening Visit will be captured as AEs on the AE eCRF page, as deemed clinically significant in the opinion of the Investigator. These abnormalities are to be followed until they reached "final outcome" (refer to Section 7.9).

6.2.3.4 Abdominal Ultrasound

To monitor subjects for liver fibrosis and potential occurrences of liver malignancies, abdominal ultrasounds will be performed. These ultrasounds will occur starting at visit Month 30 and then at each visit thereafter as specified in Table 3.

For those subjects where a long-term follow-up visit is impacted by COVID-19, abdominal ultrasounds may be conducted within the following window:

- Up to -1 month prior to the target visit
- Up to +1 month after the target visit

Adjustments to this visit schedule will be documented.

Ultrasounds will be evaluated by qualified personnel at each site for fibrosis and malignancy.

Identified abnormalities will be captured as AEs on the AE eCRF page, as deemed clinically significant in the opinion of the Investigator. These abnormalities are to be followed until they reached "final outcome" (refer to Section 7.9).

6.2.3.5 Anti-factor IX Antibodies

Anti-factor IX antibodies will be measured at the central laboratory, at baseline and at specific time points after IMP administration as specified in Table 2 and Table 4.

6.2.3.6 Factor IX Inhibitors

Factor IX inhibitors will be measured at the central laboratory with the Nijmegen modified Bethesda assay, and at the local laboratory with the Bethesda assay or Nijmegen modified Bethesda assay as specified in Table 2 and Table 4. 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 (or designee) 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.

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

If a subject is tested positive for factor IX inhibitors, a re-test should be performed preferably within two weeks to confirm the positive test. The subject should be called in for an additional visit in case no routine visit is scheduled within this two week timeframe. The subject should remain in the trial as per the clinical judgement of the Investigator.

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

6.2.3.7 Factor IX Recovery

Measurement of factor IX recovery (maximum concentration [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 factor IX will be performed at baseline (Day 0 pre IMP). Additionally, measurement of factor IX recovery and incremental recovery should be done at suspicion of factor IX inhibitor (see also Section 6.2.3.6) as judged by the Investigator.

At each occasion, a factor IX challenge dose of 40 U/kg should be administered while at the clinical trial site. A blood sample should be drawn just prior to administering the factor IX

challenge dose and at 30 minutes after the factor IX challenge dose was administered. The blood sample should preferably be drawn from a vein different from the vein used for factor IX infusion.

Date of sampling, times of blood sampling (pre and post factor IX administration), time of factor IX challenge dose administration will be recorded.

Factor IX activity for factor IX recovery assessment will be measured at a central laboratory using the one-stage aPTT assay.

6.2.3.8 Total (IgG and IgM) and Neutralizing Antibodies to AAV5

Sampling for total (IgG and IgM) and neutralizing antibodies to AAV5 will be performed at Screening, baseline and at specific time points after IMP administration as specified in Table 2 and Table 4. Total IgG and IgM antibodies will be assessed using an enzyme-linked immunosorbent assay (ELISA) and a luciferase-based assay will be used for neutralizing antibodies to AAV5. The measurements will be performed at the central laboratory. Further details of the assays will be provided in the laboratory manual.

6.2.3.9 AAV5 Capsid-specific T cells

Sampling for AAV5 capsid-specific T cells will be performed at all visits up to Week 52.

AAV5 capsid-specific T cells will be measured at the central laboratory.

6.2.3.10 Vector Genome Detection

Sampling of blood and semen to determine vector DNA levels will be performed at baseline and at specific time points post-baseline, as specified in Table 2 and Table 4 by means of quantitative (real-time) polymerase chain reaction (qPCR). Sampling should continue for the individual subject and for a specific matrix until three consecutive negative samples have been detected for the subject for that particular type of matrix. The sampling schedule may be increased (in frequency) as agreed between the Investigator and subject following the notification of a first negative result on blood and/or semen, expediting the opportunity to reach three consecutive negative samples on the specific matrix.

Based on the wish of the subject semen samples can be collected at home prior to attending the visit (at the visit day or at the day before the visit day). Also, the frequency of semen sampling may be reduced (to be agreed between Investigator and subject) as long as the subject uses a condom during sexual intercourse until three consecutive negative samples have been detected. In case a subject is not able to provide semen samples due to a medical condition, this should be recorded by the Investigator in the subjects' medical record.

6.2.3.11 Inflammatory Markers

Blood samples will be taken at all visits up to Week 52 to assess IL-1β, IL-2, IL-6, IFNγ, and MCP-1 using ELISA. All assessments will be performed at the central laboratory.

6.2.3.12 Other Safety Laboratory Evaluations

All clinical laboratory assays will be performed according to the laboratory's normal procedures. Reference ranges are supplied by the laboratory and used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The Investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical condition may be, at the discretion of the Investigator or Sponsor, repeated until confirmed, explained, or resolved as soon as possible.

The safety laboratory assessments that will be performed at the central and/or local laboratory are specified in Table 8, and as indicated in the schedule of events (Table 2 and Table 4).

Table 8 Safety Laboratory Parameters Assessed at the Central and/or Local Laboratory

Central Laboratory	
Serum Chemistry	Serum electrolytes (sodium, potassium), creatinine, creatine kinase, gamma-glutamyltransferase, AST, ALT, ALP, CRP, albumin, total bilirubin, glucose (non-fasting)
Hematology	Hemoglobin, hematocrit, platelet count, red blood cells, white blood cells with differential count, CD4+ count (all expressed in % as well as in absolute numbers)
Coagulation	aPTT, PT (or INR [International Normalized Ratio])
Serology	HIV viral load, HBsAg, HBeAg, HBV DNA and HCV RNA
Alpha-fetoprotein	AFP
Local Laboratory	AST and ALT

Abbreviations: AFP: alpha-fetoprotein; ALP: alkaline phosphatase; ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; CRP: c-reactive protein; DNA: deoxyribonucleic acid; HbeAg: hepatitis B extracellular antigen; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HCB: hepatitis C virus; HIV: human immunodeficiency virus; RNA: ribonucleic acid.

The Investigator should attempt to arrange with the local laboratory that analysis results are provided on the same day, or the day after, blood sampling has taken place. Local laboratory results should be provided as soon as possible to the Investigator.

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.

Abnormalities identified at the Screening will be documented in the subject's source documents and on the medical history eCRF. Changes after the Screening will be captured as AEs on the AE

eCRF page, as deemed clinically significant in the opinion of the Investigator. These abnormalities are to be followed until they reached "final outcome" (refer to Section 7.9).

6.2.4 Others

6.2.4.1 Blood Sample for Future Research

Four additional blood samples for the purpose of potential future research in the hemophilia B disease area (including development and validation of assays to support efficacy assessments) will be drawn (at screening, baseline [Day 0 pre IMP], Week 12, and Week 52).

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.

6.2.4.2 Liver Sample for Future Research

The Sponsor will also provide an optional consent to ask subjects to agree with providing a tissue sample from their liver in case of death, or if the liver becomes available for any other reason (e.g., liver transplantation or resection) during the long-term follow-up phase of this study. Liver samples will be analyzed to investigate how the gene therapy sequences are maintained within the cells of the liver over time, tolerance and/or stress within the cells of the liver, and/or how the gene therapy is expressed in different parts of the liver and across the liver cells. This is entirely voluntary, and subjects may still participate in the study if they do not wish to agree to donate a liver tissue sample.

6.2.5 General Information Regarding Laboratory Sampling and Results

All laboratory assessments will be conducted at a central laboratory, except the factor IX activity assay for local monitoring of subjects (Section 6.2.2.3), factor IX inhibitor assay for local monitoring of subjects and eligibility check (Section 6.2.3.6), and local monitoring of liver enzymes (AST and ALT) (Section 6.2.3.12; also see the schedule of events [Table 2 and Table 4]).

Dates and times of sampling will be recorded.

Detailed procedures for the collection, processing, storage and shipment of central 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 analyzed, they will remain stored for potential re-analysis at any time during the trial to a maximum of up to one year after the trial has been completed, before being destroyed. Where allowed by local regulations, left over material of these samples may be used throughout the study to support studies in hemophilia B and/or gene therapy research, as well as related assay development to support such research. At maximum 1 year after the study has been completed, all sample material will be 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 from Week 52 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. The Additional Visit should preferably take place within 1 week after the report of the erroneous result(s).

6.2.6 Volume of Blood to be Drawn From Each Subject

Overall, a total of approximately 2425 mL blood will be drawn from each subject during this trial (excluding additional visits). A maximum of 110 mL will be taken per visit. A maximum amount of 100 mL will be drawn per additional visit.

Approximately 2050 mL blood will be drawn as of screening and up till Week 52. During the long-term follow-up, approximately 375 mL blood will be drawn.

The amount of blood to be taken for each assessment may vary according to the instructions given in the laboratory manual. The overall total amount of blood that will be drawn from each subject may vary according to the amount of additional visits needed for the individual subject. When multiple assessments need to be done at the same time point/visit, and they require the same type of tube, the assessments may be combined.

7 SAFETY DEFINITIONS, REPORTING AND FOLLOW-UP

7.1 Adverse Event Definitions

An AE, an adverse drug reaction (ADR), and a 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 unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the IMP including the IMP administration procedure. The definition also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

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-subject 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)

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is an unexpected adverse reaction that at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.

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 Screening, 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.

7.2 Adverse Events Qualifying for Special Notification

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

- AEs related to the IMP administration procedure
- 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)
- AEs related to mandatory concomitant medication (e.g., immunosuppression)
- AEs related to medical devices which form part of the product or are used for application of the product
- Development of any new/recurrent cancer.

These AEs should be reported and followed in the same manner as SAEs. Note that the AEs may be serious or non-serious by definition (see Section 7.1).

7.3 Adverse Event Assessment Definitions

7.3.1 Severity

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

- **Mild:** A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Moderate:** A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.
- **Severe:** A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

For reporting of S(AE) related laboratory abnormalities, the severity (intensity) needs to be evaluated in accordance with the defined criteria for assessment of laboratory value abnormalities.

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.

7.3.2 Relationship to IMP

The Investigator must assess the causal relationship of the IMP for each (S)AE. The Investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the IMP according to the following guidelines and must document the causality assessment in the source document.

Term	Relationship	Definition
Related	Yes	The temporal relationship between the event and the administration of the IMP is compelling and follows a known or suspected response pattern to that product; the response disappears or decreases on cessation or reduction of the IMP dose and/or it reappears or worsens when the IMP is administered.
Possibly Related	Yes	The temporal relationship between the event and the administration of the IMP is compelling and/or follows a known or suspected response pattern to that product, but the event could reasonably be explained by the subject's medical condition, other therapies, or accident.
Unlikely Related	No	The temporal relationship between the event and the administration of the IMP is less compelling and/or does not follow a known or suspected response pattern to that product; the event could plausibly be explained by the subject's medical condition, other therapies, or accident.
Not Related	No	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the IMP and the event. In addition, this assessment can be used in cases where the subject did not receive any treatment with IMP.

7.4 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. 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.

7.5 Prompt Reporting of SAEs and Other Events to CSL Behring

SAEs, AEs qualifying for special notification, pregnancies and occupational exposure must be reported as described in Table 9 (once the Investigator determines that the event meets the protocol definition for that event).

Table 9 Timing of Reporting and Follow-up for (Serious) Adverse Events, Adverse Events for Special Notification, Pregnancies and Occupational Exposure

	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 [a]	SAE form
All AEs qualifying for special notification as defined in Section 7.2	24 hours	SAE form	72 hours [a]	SAE form
All pregnancies	24 hours	Pregnancy reporting form	Once separate informed consent is given by the subject and pregnant partner, within 72 hours [a]	Pregnancy reporting form
All occupational exposure (with or without AE)	24 hours	Occupational exposure form	72 hours	Occupational exposure form

[a.] 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 after initial report).

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

The reporting form and all other relevant documents supporting the reported SAE, AE qualifying for special notification, pregnancy or occupational exposure (e.g., diagnostic procedures, hospital records, and autopsy reports) must be reported to CSL Behring.

The Independent Ethics Committees/Institutional Review Boards (IECs/IRBs) and regulatory authorities will be notified of (S)AEs 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).

7.6 Regulatory Reporting Requirements for SAEs and Other Events

Prompt notification by the Investigator to CSL Behring of SAEs and AEs qualifying for special notification, pregnancy and occupational exposure is essential, so that legal obligations and ethical responsibilities towards the safety of subjects are met.

CSL Behring has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. CSL Behring will comply with ICH/FDA/EMA and country-specific regulatory requirements relating to safety reporting to the regulatory authority, IECs/IRBs, and Investigators.

An Investigator who receives a SUSAR describing (an) SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from CSL Behring will acknowledge, review, and file it in the appropriate section of the ISF and will notify the IECs/IRBs, if appropriate, according to local requirements (this information will also be filed in the appropriate ISF section by the Investigator).

In addition to submission of SAEs, an annual development safety update report will be prepared and submitted to the EMA, FDA, and locally if applicable, according to the development international birth date.

7.7 Reporting of Pregnancies

Should a pregnancy occur in a female partner of male subject, it 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 follow 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 by the subject's pregnant partner/infant's mother.

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.

7.8 Reporting of Occupational Exposure

Occupational exposure refers to the exposure to the IMP 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.

7.9 Follow-up on Adverse Events

All S(AE)s should be followed until resolved or they have reached a "final outcome" or the subject's participation in the trial ends, whichever comes first.

Severe, non-serious AEs assessed as "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).

8 DATA MANAGEMENT

8.1 Data Collection

The Investigators' authorized site personnel must enter the information required by the protocol on the eCRF (note that authorized personnel from the data management vendor will enter the CCI and CCI summary data). A trial monitor will visit each site or perform virtual monitoring in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered by the site on the eCRF will be addressed by qualified site personnel or the data management vendor for CCI and CCI summary data. When a data discrepancy warrants correction, the correction will be made by authorized site personnel or by authorized personnel from the data management vendor, where applicable. Data collection procedures will be discussed with the site personnel at the site initiation visit and/or at the Investigator's Meeting. It is expected that site personnel will complete the eCRF entry within 5 business days of the subject's visit.

The subject reported e-diary data will be directly loaded from the application into the eCRF up to Week 52, without source documentation. All other data will have separate source documentation; these data will not be recorded directly onto the eCRF.

8.2 Clinical Data Management

Data are to be entered into the eCRF as specified in data entry instruction. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

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.

8.3 Study Data

Study data identified in this protocol are collected, and source verified, on eCRF. All study data will be formulated into data sets to provide transparency, traceability, and integrity of trial analysis results from collection source to meet regulatory obligations for standardized study data. Observed study data will be mapped to the CDISC Study Data Tabulation Model (SDTM) and serve as the source data from the trial. Study analyses will be completed using analysis data sets that are derived from the SDTM and follow the CDISC Analysis Data Model (ADaM) architecture.

8.3.1 Clinical Data – CDISC Study Data Tabulation Model (SDTM)

Domains will be mapped to CDISC SDTM version 3.2. No derived data required for analysis are included in the SDTM domains. All SDTM domains will be fully documented with define documents (DEFINE.XML) and a reviewer's guide after database lock and final analyses are completed.

8.3.2 Analysis Data – CDISC Analysis Data Model (ADaM)

Planned and CCI [redacted] will be completed using the ADaM data sets derived from the SDTM domains for this study. Analysis data sets will contain all derived study endpoints required for analysis. All analysis data sets will be fully documented with define documents (DEFINE.XML) and a reviewer's guide after database lock and final analyses are completed.

9 STATISTICAL METHODS

9.1 Statistical Analysis

Details regarding the statistical methods and definitions will be provided in the statistical analysis plan (SAP), and will include templates for the tables, figures and listings to be provided.

The SAP will be finalized before the interim analysis.

Any agreed deviations from the SAP are to be justified in the clinical trial report.

Statistical analyses will be performed using SAS® Version 9.4 or higher (SAS Institute, Cary, NC 27513).

Considering the small sample size used in this trial, all presentations will be descriptive in nature. No formal, inferential statistical analyses will be performed. Plots, tabular displays, and listings will be created, visualizing individual effects for selected efficacy and safety measures. If applicable, continuous variables will be summarized with descriptive statistics including: the number of non-missing values, mean, standard deviation, median, minimum, and maximum. In some cases, the standard error of the mean and/or confidence intervals may be presented. Categorical variables will be summarized by number, percent of subjects and, if applicable, the number of events. No formal inferential statistical analyses will be performed, and no analysis population will be defined. All available data will be used in the presentations.

9.2 Planned Interim Analysis

At six weeks after administration of AMT-061, a data cleaning round will be performed to provide data for one interim analysis for efficacy using the available data on the primary efficacy parameter - factor IX activity at six weeks. The rationale for the six weeks cut-off is based on data from the Phase 1/2 trial that demonstrated a very fast initial transduction of AAV5 and expression of the endogenous factor IX protein within the first week, which reached a relatively stable peak level within the first six weeks of follow-up. The results will be reported in an interim Clinical Study Report (iCSR).

Furthermore, for this interim analysis, plots, tabulations, and/or listings will be provided for other efficacy and safety data collected at six weeks (as detailed in the SAP), to provide additional information on the factor IX activity levels.

9.3 Full Analyses

After 52 weeks post-dose, and 2.5 years post-dose, efficacy and safety data will be collected on all subjects. The data will be locked, analyzed, and reported in a CSR (52-week analysis) and a CSR addendum (2.5-year analysis).

At the end of the 4-year long-term follow-up period (60 weeks/5 years post-dose), all safety and efficacy data will be reported in a CSR addendum (5-year analysis).

The CSR and CSR addenda will 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.

9.4 Selection of Subjects to be Included in the Analyses

No formal analysis population is defined. All available data on treated subjects will be included in the efficacy and/or safety analysis of the data.

9.5 Subject Disposition

The data on subject disposition and informed consent will be listed, containing details on treated and completed subjects, as well as information regarding withdrawal reasons or protocol deviations (including those related to COVID-19), if applicable.

9.6 Demographic and Baseline Characteristics

Descriptive summaries of demographic and baseline characteristics will be presented. The following descriptive statistics will be included: number of observations, mean, standard deviation, median, minimum, and maximum for quantitative data. For qualitative data, frequency counts and percentage will be determined.

Other baseline data will only be listed.

Medical history will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA) and listed for each subject.

9.7 Investigational Product Exposure

Listings for exposure to IMP will be presented.

9.8 Prior and Concomitant Medication

Prior and concomitant medications will be coded using the most recent World Health Organization drug dictionary. Prior and concomitant medications will be listed.

9.9 Efficacy Analyses

All efficacy analyses will be performed for all treated subjects. All presentations will be done on an individual level or by using descriptive statistics. Plots and tabular displays will be created, visualizing individual effects for the selected efficacy measures as specified in the following sections. Analyses will be based on central laboratory measurements if results are available from both local and central laboratories.

9.9.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the factor IX activity level at six weeks after dosing.

Factor IX activity levels will be presented in % of normal units. Descriptive statistics will include mean, standard deviation, median, minimum, and maximum over time for per subject factor IX activity levels presented at six weeks after dosing by subject and also by visit across subjects. Subject plots of factor IX activity levels over time will be presented. Descriptive statistics and plots will only display uncontaminated results, i.e., factor IX activity levels that are not affected by exogenous factor IX use during the trial. The required wash out period (in order to consider a factor IX activity level to be "unaffected" – i.e., "uncontaminated") will be 10 days.

Listings will present all factor IX activity levels measured, and will include a flag for contaminated results.

9.9.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints comprise of factor IX activity at 52 weeks, bleeding rates (including a further break down of the reported bleeding episodes) and usage of factor IX replacement therapy. Data will be presented using descriptive statistics. Plots of selected endpoints will be provided. All data will also be listed.

CCI

CCI

[redacted]

9.10 Safety Analyses

All treatment-emergent AEs are tabulated by SOC and preferred terms within each SOC according to the MedDRA terminology list. TEAEs will also be tabulated by severity (mild/moderate/severe using the Common Terminology Criterion for Adverse Events severity

grades) and by relationship (related/not related) to trial medication, using frequency counts (number of subjects with event and number of events) and percentages. Similar tables will be created for TEAEs leading to premature discontinuation, deaths, and SAEs, if applicable.

These summary tables will be presented by decreasing frequency of occurrence based on SOC and Preferred Term.

An AE overview table will be created displaying the number of subjects (and percentage) experiencing an event and the number of events for: Any TEAE, mild/moderate/severe TEAE, related/unrelated TEAE, serious TEAE, TEAE for special notification, TEAE leading to discontinuation.

The summary tables will be accompanied by individual subject listings of all AEs, including information on AE number, actual AE description, date/time of start and end of AE, preferred term (MedDRA), SOC (MedDRA), severity, relationship/causality, type of AE, seriousness and outcome. Pre-existing AEs will be flagged. Pre-existing AEs are not considered to be treatment-emergent, except in case of worsening during/after trial treatment (to be collected as separate AE). Separate listings will be created for TEAEs for special notification, deaths, and SAEs, if applicable.

Summary statistics for the baseline assessment and change from baseline at each post-baseline visit for scheduled lab assessments of continuous laboratory variables will be tabulated. Clinically significant laboratory values will be tabulated. Clinical laboratory tests will be listed and summarized by visit, with reference range and flag indicating if the measurement in question is outside the reference range provided.

Summary statistics of the absolute value and change from baseline for vital sign measurements will be tabulated by visit, and time point. Vital sign measurements will be listed.

All data for anti-factor IX antibodies, factor IX inhibitors, factor IX recovery, total (IgG and IgM) and neutralizing antibodies to AAV5, AAV5 capsid-specific T-cells, inflammatory markers, vector DNA in semen and blood, will be listed. Plots of IgM and IgG neutralizing antibodies, AAV5 capsid-specific T cells, and vector DNA in semen and blood over time will be provided.

9.11 Sample Size Justification

There is no formal sample size calculation performed for this trial. From a clinical perspective, N=3 subjects is considered sufficient to provide a reliable impression of the factor IX activity levels and safety profile that will be demonstrated with a single dose of 2×10^{13} gc/kg AMT-061 at six weeks after dosing.

9.12 Data Monitoring Committee

A data monitoring committee (DMC) will be involved in the management of the overall AMT-061 program including this clinical trial. The purpose of the DMC in this trial is to monitor safety of the subjects in the trial as well as to evaluate response to the treatment in terms of factor IX activity levels. The DMC will assess whether observed factor IX activity levels are within an expected range to determine if the administered dose is suitable for administration in a pivotal phase 3 trial (range to be defined in DMC charter). If the DMC determines that the observed response is not within the expected range, or they do not observe enough consistency of effect, to proceed to Phase 3 dosing they can elect to recommend up to three more subjects be treated at the same dose or recommend a second dose be studied. DMC meetings will be held at set times during the trial as outlined in the DMC charter. Further details regarding the DMC can be found in the DMC charter, which will be available prior to the administration of IMP.

10 SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This trial is conducted in accordance with current applicable regulations, ICH GCP (E6[R2]), EU Directive 2001/20/EC, EU Directive 2005/28/EC, FDA guidelines, ATMP guidelines and its updates, and local ethical and legal requirements.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The trial sponsor and any third party to whom aspects of the trial management or monitoring have been delegated will undertake their assigned roles for this trial in compliance with the ICH GCP Guideline E6(R2), and related detailed guidelines specific to ATMPs (ENTR/F/2/SF/dn D(2009) 35810), as well as with applicable regulatory requirements in the countries where the trial will take place.

Representatives of the trial sponsor, and/or the company organizing/managing the research on behalf of the Sponsor, conduct visits to sites to inspect trial data, subjects' medical records, and eCRFs in accordance with current ICH GCP and the respective local and inter/national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The Sponsor ensures that Local Regulatory Authority requirements are met before the start of the trial. The Sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any Regulatory Authority and IRB/IEC approvals required prior to release of IMP for shipment to the site.

10.1.2 Indemnity/Liability and Insurance

The Sponsor ensures that a fully executed contract is in place prior to initiation of the trial, including appropriate wording on indemnification of the Investigator/the institution against claims arising from the trial, as per applicable regulations.

The Sponsor ensures that suitable clinical trial insurance coverage is in place prior to the start of the trial. An insurance certificate is supplied to the Investigator as necessary.

10.1.3 Public Posting of Trial Information

The Sponsor will assure that key design elements of this protocol will be posted in a publicly accessible database such as Clinical Trials.gov, prior to the start of the trial. In addition, upon trial completion and finalization of the trial report the results of this trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results. Information included in clinical trial registries may include participating Investigators' names and contact information.

The Sponsor will retain ownership of all data.

10.1.4 Submission of Summary of Clinical Trial Report to Competent Authorities of Member States Concerned and Independent Ethics Committees

The Sponsor will provide a summary of the CSR within 1 year of the end of the trial completion date to the competent authority of the Member State(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on ICH GCP. The Sponsor will provide the IRBs/IECs with a copy of the same summary.

10.1.5 Trial Suspension, Termination, and Completion

The Sponsor may suspend or terminate the trial or part of the trial at any time for any reason. If the trial is suspended or terminated, the Sponsor will ensure that the Investigator as well as applicable regulatory agencies and IRBs/IECs are notified as appropriate. Additionally, the discontinuation of a registered clinical trial will be published on the publicly available database (as applicable).

The Sponsor will make an end-of-trial declaration to the relevant competent authority as required by Directive 2001/20/EC.

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The Investigator must agree to conduct the trial in accordance with ICH GCP Guideline E6, including related detailed guidelines specific to ATMPs and applicable regulatory requirements and guidelines.

It is the Investigator's responsibility to ensure that adequate time and appropriate trained resources are available at the site prior to commitment to participate in this trial. The Investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The Investigator will maintain a list of appropriately qualified persons to whom the Investigator has delegated significant trial-related tasks. *Curriculum vitae* for Investigators and sub-investigators are provided to the trial Sponsor (or designee) before starting the trial.

If a potential research subject has a primary care physician, the Investigator should, with the subject's consent, inform him or her of the subject's participation in the trial.

An international coordinating Investigator is appointed to review the final Clinical Trial Report. Agreement with the final Clinical Trial Report is documented by the signed and dated signature of the coordinating Investigator, in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

10.2.2 Protocol Adherence and Investigator Agreement

The Investigator and any sub-investigators must adhere to the protocol as detailed in this document. The Investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an Investigator Agreement to confirm acceptance and willingness to comply with the trial protocol.

If the Investigator suspends or terminates the trial at his or her site, the Investigator will promptly inform the Sponsor, the IEC/IRB, and, where applicable, the regulatory authority, and provide them with a detailed written explanation. If the trial is prematurely terminated or suspended for any reason, the Investigator will promptly inform the trial subject(s) and will assure appropriate medical care and follow-up for the subjects. The Investigator will also return all IMPs, containers, and other trial materials to the Sponsor. Upon trial completion, the Investigator will provide the Sponsor, IEC/IRB, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IECs/IRBs, to ensure accurate and timely information is provided at all phases during the trial, may be done by the Sponsor, applicable Contract Research Organization (CRO), Investigator, or for multi-site trials, the coordinating Investigator according to national provisions and will be documented in the Investigator Agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Electronic Case Report Forms

Access to the eCRF system and data capture by the Sponsor/CRO should be supported, and should be handled in accordance with instructions from the Sponsor.

The Investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. Electronic case report forms must be completed by the Investigator or designee as stated in the site delegation log (note that authorized personnel from the data management vendor will enter CCI and CCI summary data on the appropriate eCRF pages).

The subject reported e-diary data will be directly loaded from the application into the eCRF, without source documentation. All other data will have separate source documentation; will not be recorded directly onto the eCRF.

All data sent to the Sponsor must be endorsed by the Investigator.

The clinical research associate (CRA)/Trial Monitor will verify the contents against the source data per the Monitoring Plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

CCI

The CCI include the patient (CCI) questionnaires. It also includes the e-diary in which the subjects will record their use of factor IX replacement therapy and bleeding episodes.

10.2.3.3 Recording, Access, and Retention of Source Data and Trial Documents

Original source data to be reviewed during this trial will include, but are not limited to: subject's medical file, original clinical laboratory requisition forms and reports, CCI summary score sheets, CCI and data available from the subjects e-diary device.

All key data must be recorded in the subject's medical records.

The Investigator must permit authorized representatives of the Sponsor, the respective national, local, or foreign regulatory authorities, the IEC/IRB, and auditors to inspect facilities and to have direct access to original source records relevant to this trial, regardless of media.

The CRA/Trial Monitor (and auditors, IEC/IRB or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the Sponsor or its representatives, national or local regulatory authorities, or the IEC/IRB having access to source data (e.g., subject's medical file, appointment books, original laboratory reports, X-rays etc.). Non-trial site personnel will not disclose any personal information or personal medical information.

These records must be made available within reasonable times for inspection, if required, by a properly authorized representative of any regulatory agency (e.g., the US FDA, EMA, UK Medicines and Healthcare products Regulatory Agency [MHRA]) or other).

Essential documents must be maintained according to local regulations or ICH GCP (E6[R2]) requirements, whichever is longer. Essential documents may not be destroyed without written permission from the Sponsor. Note that traceability records should be kept for a minimum of 30 years after the expiry date of the product, or longer if required by the terms of the clinical trial authorization or by the agreement with the Sponsor.

10.2.3.4 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this trial must be available for inspection upon request by representatives of, for example, the US FDA (as well as other US national and local regulatory authorities), the EMA, the MHRA, other regulatory authorities, the Sponsor or its representatives, and the IEC/IRB for each site.

10.2.3.5 Financial Disclosure

The Investigator is required to disclose any financial arrangement prior to participating in the trial and for up to one year after, whereby the value of the compensation for conducting the trial could be influenced by the outcome of the trial.

The Investigator should provide the following information: any significant payments from the Sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in IMP; any significant equity interest in the Sponsor or subsidiaries as defined in 21 Code of Federal Regulations (CFR) 54 2(b) (1998).

In consideration of participation in the trial, the Sponsor pays the Investigator or nominated payee the sums set out in the payment schedule attached to the Investigator agreement.

10.2.4 Compliance to all Regional, Local, State, and National Controlled-substance, Biohazard and Infectious Disease Regulations and Legislation

When using controlled substances, biohazardous material, or substances for infectious diseases, the Investigator must at all times comply with all regional, local, state, and national laws pertaining to registration, reporting with the appropriate regulatory body and control and handling of such substances.

10.3 Ethical Considerations

10.3.1 Informed Consent

It is the responsibility of the Investigator, or designee, to obtain voluntary written informed consent from all trial subjects prior to any trial related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations, the GCP specific to ATMPs (ENTR/F/2/SF/dn D(2009) 35810) and ICH GCP (E6[R2]). In accordance with the ATMP guideline, the informed consent form and any other written information should include an explanation of particular issues associated with ATMPs, e.g., inconveniences of long-term follow-up, specific risks such as shedding and the irreversible nature of the ATMP and other issues as further listed in the guideline.

Each subject is requested to sign the Subject Informed Consent Form in their local language, after the subject has received and read (or been read) the written subject information and received an explanation of what the trial involves, the particular issues arising with ATMPs, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities.

If the ICF is revised with important new information that must be shared with the study subjects, the amended ICF will be presented, as required by the IRB/IEC for review and consideration by the subject, and signed re-consent is to be obtained.

Separate written informed consents are to be obtained for the factor IX gene sequencing and future research samples prior to collection of the samples, as detailed in Section 6.2.1.6 and Section 6.2.4.1, respectively.

A copy of all informed consent documentation (i.e., a complete set of subject information sheets and fully executed signature pages) must be given to the subject. If applicable, it is provided in a certified translation of the local language. Signed consent forms must remain in each subject's trial file and must be available for verification at any time.

The principal Investigator provides the Sponsor with a copy of the local consent form which was reviewed by the IEC/IRB and which received their favorable opinion/approval. A copy of the IEC/IRB's written favorable opinion/approval of these documents must be provided to the Sponsor, prior to the start of the trial unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to trial start that another party (i.e., Sponsor or coordinating Investigator) is responsible for this action. Additionally, if the IEC/IRB requires modification of the sample Subject Information and Consent document provided by the Sponsor, the documentation supporting this requirement must be provided to the Sponsor.

The Sponsor will also provide an optional consent to ask subjects to agree with providing a tissue sample from their liver in case of death, or if the liver becomes available for any other reason (e.g., liver transplantation or resection) during the long-term follow-up phase of this study. Liver samples will be analyzed to investigate how the gene therapy sequences are maintained within the cells of the liver over time, tolerance and/or stress within the cells of the liver, and/or how the gene therapy is expressed in different parts of the liver and across the liver cells. This is entirely voluntary, and subjects may still participate in the study if they do not wish to agree to donate a liver tissue sample.

10.3.2 Institutional Review Board or Independent Ethics Committee

For sites outside the EU, it is the responsibility of the Investigator to submit this protocol, the informed consent document (approved by the Sponsor or its designee), relevant supporting information and all types of subject recruitment information to the IECs/IRBs for review, and all must be approved prior to site initiation.

For sites within the EU, the applicant for an IEC opinion can be the Sponsor, the Investigator, or for multi-site trials the coordinating Investigator or Sponsor, according to national provisions.

Responsibility for coordinating with IECs/IRBs is defined in the Investigator Agreement.

Prior to implementing changes in the trial, the Sponsor and the IECs/IRBs must approve any revisions of any revised informed consent documents and amendments to the protocol unless there is a subject safety issue (in that case approval can be after implementation).

Investigational product supplies will not be released until the Sponsor has received written IECs/IRBs approval of and copies of revised documents.

For sites outside the EU, the Investigator is responsible for keeping the IECs/IRBs appraised of the progress of the trial and of any changes made to the protocol, but in any case at least once a year. For sites within the EU, this can be done by the Sponsor, the Investigator or for multi-site trials the coordinating Investigator, according to national provisions. The Investigator must also keep the local IECs/IRBs informed of any serious and significant AEs.

The names of the IECs/IRBs chairperson and the members of the IECs/IRBs will be collected as well as a statement that the IECs/IRBs works in accordance with the principles of ICH GCP.

10.3.3 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

10.4 Privacy and Confidentiality

All US-based sites and laboratories or entities providing support for this trial, must, where applicable, comply with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). A site that is not a Covered Entity as defined by HIPAA, must provide documentation of this fact to the Sponsor.

All EU-based sites and laboratories or entities providing support for this trial, must, where applicable, comply with the Data Protection Directive 95/46/EC (24 Oct 1995), EU data protection regulations No. 45/2001 (18 Dec 2001), and EU General Data Protection Regulation (GDPR) 2016/679 (27 Apr 2016).

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the trial, the Sponsor and/or its representative reviews their medical records and data collected during the trial. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the Sponsor; third parties with whom the Sponsor may develop, register, or market AMT-061; national or local regulatory authorities; and the IRBs/IECs, which gave

approval for the trial to proceed. The Sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number. However, age and birth year may be collected and used to assist the Sponsor to verify the accuracy of the data, for example, to confirm that laboratory results have been assigned to the correct subject.

The results of trials – containing subjects' unique identifying number, relevant medical records, and possibly age and birth year – will be recorded. They may be transferred to, and used in, other countries that may not afford the same level of protection that applies within the countries where this trial is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the trial results, or to answer questions asked by regulatory or health authorities.

10.5 Publication Policy

All manuscripts, abstracts, or other modes of presentation arising from the results of the trial must be reviewed and approved in writing by the Sponsor, in advance of submission. The review is aimed at protecting the Sponsor's proprietary information either existing at the date of the commencement of the trial, or generated during the trial. Authorship will follow guidelines established by the International Committee of Medical Journal Editors (ICMJE, 2015). The publication policy with respect to the Investigator and clinical trial site will be further detailed in a separate document.

11 ADMINISTRATIVE ASPECTS

11.1 Investigator(s)

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 will be appointed for each participating country. This Investigator will be responsible for national issues relating to the trial.

Responsibilities of the Investigator are described in Section 10.2.

11.2 International Coordinating Investigator

The International Coordinating Investigator is responsible for approval of the clinical trial report on behalf of all trial Investigators.

11.3 Clinical Trial Sites

Clinical trial sites should also have immediate access to equipment and staff for resuscitating and stabilizing 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.

11.4 Vendors

Trial management, vendor oversight, and medical monitoring oversight will be provided by uniQure biopharma B.V.

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, and manage central preparation and storage of samples.
- Clinical monitoring and project management
- Regulatory
- Medical Monitoring
- Data Management
- DMC Administration
- Statistics

- Medical Writing
- The provision of the CCI
- The provision of the e-diaries
- eTMF
- Investigator Meeting organization
- Subject travel organization and reimbursement

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13 APPENDICES

13.1 Laboratory Information

Laboratory	Test/Profile
PPD PPD	Central Laboratory: Coagulation Chemistry Inflammatory markers/cytokines Serology Hematology CD4 counts PBMC isolation for AAV5-capsid specific T-cell response
PPD PPD	Referral testing: Vector genome detection (blood and semen) Neutralizing antibodies to AAV5
PPD PPD	Referral Testing: FIX Activity: one-stage aPTT, chromogenic assay FIX protein concentration FIX inhibitors Anti-FIX antibodies IgG antibodies AAV5/IgM antibodies AAV5
PPD PPD	Referral Testing: AAV5-capsid specific T-cell
PPD PPD	Referral Testing: FIX genetic sequencing
PPD PPD	Confirmation Testing: HIV-1/2 Antibody differentiation test

13.2 Summary of Changes in Current Amendment

Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number: 3.0 (Version 4.0)	**Amendment Date:** 08 Feb 2022	**Global/Country/Site-Specific:** Global
Description of Change		**Section(s) Affected by Change**
The Sponsor was changed from uniQure to CSL Behring.		All
Minor administrative text and formatting updates.		All
It was clarified that uniQure will provide oversight to Medpace, the Monitoring CRO.		Title Page
Administrative changes to the identified Sponsor representative.		Signature Page
The entity responsibile for safety oversight including SAE/AESI/pregnancy reporting was updated to CSL Behring, with details provided for contacting CSL Global Clinical Safety & Pharmacovigilance.		Emergency Contact Information Section 7.5 Section 7.6
The identity and details for the uniQure Medical Director were updated.		Emergency Contact Information
Optional consent to ask subjects to agree with providing a tissue sample from their liver in case of death, or if the liver becomes available for any other reason (e.g., liver transplantation or resection) during the long-term follow-up phase of this study was added.		Section 6.2.4.2 Section 10.3.1
It was clarified that trial management, vendor oversight, and medical monitoring oversight will be provided by uniQure. Pharmacovigilance was removed from the list of services that the Sponsor will engage vendors to perform.		Section 11.4

13.3 Previous Amendments

Summary of Changes		
Amendment Number: 2.0 (Version 3.0)	**Amendment Date:** 10 Feb 2021	**Global/Country/Site-Specific:** Global
Description of Change		**Section(s) Affected by Change**
Administration changes to identified sponsor representatives, abbreviation use, formatting, etc, for consistency.		All
Update to uniQure Medical Director details.		Emergency Contact Information
Addition of "etranacogene dezaparvovec" to the description of the IMP identity		Title Page Synopsis Section 5.1
Addition of comment that adjustments to the visit location and/or schedule may be made to accommodate safety concerns and restrictions due to the COVID-19 pandemic was added in discussion of the long-term follow-up phase.		Synopsis
Addition of a CSR Addendum for analyses after 2.5 years of follow-up post treatment.		Synopsis Section 3.1 Section 6.1.3 Section 9.3
Clarification that study-specific paper diaries are to be used during the long-term follow-up for bleeds (long-term follow-up bleed diary) and factor IX use (long-term follow-up factor IX use diary) and brought to all clinic visits in this phase for reporting. Paper diaries will be distributed at the Week 52 visit.		Synopsis Table 1 Table 3 Section 6.1.3, 6.1.4 Section 6.2.2.1, 6.2.2.2
An abdominal ultrasound to monitor subjects for liver fibrosis and potential occurrences of liver malignancy was added as a safety objective and assessment, with assessments occuring in the the long-term follow-up phase beginning at the Month 30 visit and at every visit thereafter until study completion (i.e., at Month 30, 36, 42, 48, 54, and 60). Details related to permitted visit window adjustments were included; adjustments to the visit timing within the allowed visit window are to be documented. Section 6.2.3.4 Abdominal Ultrasound was added.		Synopsis Table 3 Section 1.6 Section 2.3 Section 3.3.2 Section 6.1.4 Section 6.2.3.4

The label for the event of "Hematology parameters" was updated to "Hematology and coagulation parameters" to be more representative of the parameters being collected, as specified in the associated footnote.	Table 2 Table 4
Clarification that the sampling schedule for vector genome detection assessments can be increased (in frequency) as agreed between the Investigator and subject following the notification of a first negative result on blood and/or semen.	Table 2 Table 4 Section 6.2.3.9
Addition of a visit window for CCI to be completed for the Month 24, Month 36, Month 48, and Month 60 visits during the Long-term Follow-up Phase, if those visits are impacted by COVID-19. Adjustments to the visit timing within the allowed visit window are to be documented.	Table 3 Section 6.2.2.4
Local laboratory assessments of factor IX (one-stage aPTT for factor IX activity) and transaminases (AST/ALT) for local monitoring were added to the long-term follow-up activities (starting at Month 30 as all subjects were approaching this visit at the time of the protocol amendment). Reference to the schedule of events for laboratory parameters (Table 2 and Table 4) was added to the following sections, for clarity on the timing of these assessments during the study: Section 6.2.3.12 (Other Safety Laboratory Evaluations) Section 6.2.5 (General Information Regarding Laboratory Sampling and Results).	Table 4 Section 6.2.3.12 Section 6.2.5
Section 1.6 "Accommodations Due to the COVID-19 Pandemic" was added as an introduction to the COVID-19 pandemic and its impact on clinical trial procedures.	Section 1.6 (new)
Section 3.1.1 "Considerations due to the COVID-19 Pandemic" was added to discuss potential adjustments to the meeting schedule or visit location/method due to the COVID-19 pandemic and to indicate that all deviations from the protocol due to the pandemic are to be documented.	Section 3.1.1 (new)
Recommendations on use of antithrombotic treatment was added for subjects with factor IX activity in the non-hemophilic (≥40% of normal) range and with a confirmed COVID-19 infection.	Section 5.6.2, 5.6.3 Section 6.2.2.1

Additional clarification and recommendations on transaminase elevation management was added including discussions on corticosteroid tapering, considerations with prolonged high dose corticosteroid use, and considerations with potential and confirmed COVID-19 infections.	Section 5.6.4
Addition of phrase indicating that the 5 years of follow-up after administration of AMT-061 is in line with the FDA guidance on long-term follow-up after administration of human gene therapy products.	Section 6.1.4
Removal of use of international criteria for assessment of laboratory value abnormalities.	Section 7.3.1
To align with the eCRFs, the possible options for causality assessments for AEs were updated, with options being related, possibly related, unlikely related, and not related.	Section 7.3.2
Clarification that trial monitor visits may be virtual.	Section 8.1
Mention of update to eCRFs to include COVID-19 reasons for missed visits.	Section 8.2
Specification that the CSR and CSR addenda will 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.	Section 9.3
Specification that the protocol deviations includes those related to COVID-19.	Section 9.5
The Investigator's Brochure references were updated, to refer to the combined document for AMT-060 and AMT-061 (Version 1; February 2020)	Section 12
Section 13.3 Previous Amendments added	Section 13.3

Amendment Number: 1.0 (Version 2.0)	**Amendment Date:** 21 May 2019	**Global/Country/Site-Specific:** Global
Description of Change		**Section(s) Affected by Change**
The title page was updated to show the name of the Monitoring CRO in place of the monitor's name.		Title Page
Update of amendment version throughout. Addition of "Summary of Changes" section Addition of Section 13.1 "Summary of Changes in Current Amendment".		Throughout Summary of Changes Section 13.1

Contact information for Medpace Clinical Safety was added. The Medpace Monitor was updated to the Medpace Medical Director and contact information for the uniQure Medical Director was added, with clarification and protocol- or safety-related issues must be sent to both of these individuals.	Emergency Contact Information
FIX was changed to factor IX throughout except in the name of the IMP.	Throughout
Definition of gc was updated to genome copies from gene copies in the List of Abbreviations.	List of Abbreviations
It was clarified tht for ALT level increments of at least 2-fold baseline and > ULN, and for AST level increments of at least 2-fold ULN, the Investigator should contact the Medpace and uniQure Medical Directors to discuss a clinical management plan on a case-by-case basis, including potential re-tests and/or initiation of corticosteroid treatment. Section 5.6.4 was created as the section for Guidelines for ALT Elevations.	Synopsis Trial Schedules (Table 1) Section 5.6.4
Update to language stating that Investigators will assess each bleeding episode, not adjudicate each bleeding episode.	Synopsis Trial Schedules (Table 1) Section 6.1.2 Section 6.1.6 Section 6.2.2.2
It was clarified that CCI [redacted] being completed by the subject prior to other assessments are performed was a recommendation. Questions should be completed in the same order at each visit, following the order presented in the protocol.	Synopsis Trial Schedules Section 6.1.2.1 Section 6.1.3 Section 6.1.4 Section 6.2.2.4
Descriptions of statistical analyses were expanded, including providing a full list of objectives and endpoints in the synopsis and body. In the body, expanded descriptions included specifying how continuous and categorical variables will be summarized, addition of a 10 day required wash out period for factor IX activity levels to be considered "uncontaminated", CCI [redacted] specification that summary statistics of change from baseline measurements for lab assessments and vital sign measurements will be presented, and addition that data	Synopsis Section 2.2 Section 3.3.2 Section 9

for anti-factor IX antibodies, factor IX inhibitors, factor IX recovery, total (IgG and IgM) and neutralizing antibodies to AAV5, AAV5 capsid-specific T-cells, inflammatory markers, vector DNA in semen and blood, will be listed. Section 9.3 Final Analyses was added to separate how the final analyses will be reported compared to the interim analysis (included in Section 9.2).	
Alpha-fetoprotein (AFP) was added as an assessment (measured at Week 52 and all long-term follow-up visits), as part of the safety objective, and as a safety endpoint.	Synopsis Trial Schedules Section 3.3.2 Table 8
Text describing management of endogeneous factor IX activity was updated to always present what should be done if endogenous factor IX activity is ≥5% followed by if it is between 2 and 5% and then <2%.	Synopsis Section 5.6.2
Specification that subjects are expected to document factor IX usage and bleeding episode during the long-term follow-up, but not in the e-diary.	Synopsis Section 6.1.4 Section 6.2.2.1 Section 6.2.2.2
Clarification that analyses will be based on central laboratory measurements if results are available from both local and central laboratories.	Synopsis Section 6.2.2.3 Section 9.9
Sampling for vector genome detection from semen sample at Week 52 was added.	Trial Schedules (Table 2)
It was clarified that assessment of blood pressure, pulse, and body temperature was assessment of vital signs. Title for Section 6.2.3.2 was updated to “Vital Signs” instead of “Blood Pressure, Pulse, and Body Temperature”.	Trial Schedules Section 3.3.2 Section 6.2.3.2
It was clarified that study visits should be scheduled to ensure they take place when factor IX activity is at its trough, on the day that routine prophylactic factor IX replacement treatment is planned to be administered.	Trial Schedules Section 6.2.2.3
Addition of a chromogenic assay for factor IX activity assessments. Specification that no additional blood will be drawn for this; tests will be performed on back-up.	Trial Schedules (Table 2 and Table 4) Section 6.2.2.3 Section 6.2.3.6
Update on the number of people with hemophilia B and the number of live male newborns with haemophila B based on updated references.	Section 1.1

Details for the second and last reference listed in Table 5 were updated.	Table 5
Section 1.5 was updated to align with the text in the related CT-AMT-061-01 protocol amendment dated 07 Dec 2018. The title of Section 1.5.2 was updated to refer to AAV5 Gene Therapeutic Vector rather than AAV5 Gene Transfection.	Section 1.5
Clarification that transaminase increase alone may warrant the initiation of a corticosteroid was made in Section 1.5.3, for consistency with Section 5.6.4.	Section 1.5.3
Addition of "Withdrawal by principal Investigator" as a possible reason for discontinuation.	Section 4.5.1
CCI [redacted]	Section 5.2 Table 6
Section 5.6.3 Guidelines for Use of Factor IX for Patients Undergoing Major Surgery was added.	Section 5.6.3
For assessment of endogenous factor IX activity, the upper factor IX activity limit was set to >5% for consistency with the other limits of 2-5% and <2%.	Section 6.1.4
Information on what will be collected for concomitant medications/therapys was updated to align with the study eCRFs.	Section 6.2.1.3
Information on what will be collected for medical history related to hemophilia B was updated to align with the study eCRFs.	Section 6.2.1.4
Information on what will be collected with respect to factor IX replacement therapy was updated to align with the e-diary questions.	Section 6.2.2.1
Information on what will be collected with respect to bleeding episodes was updated to align with the e-diary questions. Definitions for joint and muscle bleeds were removed. The definition for persistent bleed was added.	Section 6.2.2.2
Clarification that local laboratory results should be provided to the Investigator as soon as possible.	Section 6.2.3.11

Section 7.3.2 Relationship to IMP was updated to reflect what is actually being done for assessment of (S)AE causality, using terms of definitely related, probably related, possibly related, and not related rather than related or not related.	Section 7.3.2
Clarification that consent of the pregnant partner is required to follow any pregnancies and outcomes.	Section 7.7
Clarification that authorized personnel from the data management vendor will enter the CCI and CCI summary data and that subject reported e-diary data will be directed loaded into the eCRF without source documentation was added.	Section 8.1 Section 10.2.3.1
Section 8.3 Study Data was added to be reflective of what is being done.	Section 8.3
Language around indemnity/liability and insurance was updated.	Section 10.1.2
Clarification and re-consent will be necessary if the ICF is revised with important new information.	Section 10.3.1
Appendix on Laboratory Information was added.	Section 13.1

Signature Page

CT-AMT-061-01 - Protocol Amendment - 3 - 08Feb2022

Signed By	Date (GMT)
PPD	PPD
Approved-PPD Approval	
PPD	PPD
Approved-Clinical Development Physician Approval	
PPD	PPD
Approved-PPD Approval	
PPD	PPD
Approved-PPD (or delegate) Approval	

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