CSL Behring

# CLINICAL TRIAL PROTOCOL

| | |
|---|---|
| Trial Title: | Phase III, open-label, single-dose, multi-center multinational trial investigating a 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: | Phase III trial of AMT-061 in subjects with severe or moderately severe hemophilia B. |
| Protocol Identification: | CT-AMT-061-02 |
| EudraCT No.: | 2017-004305-40 |
| Name of Investigational Product: | AMT-061 (AAV5-hFIXco-Padua; etranacogene dezaparvovec) |
| IND No. | CCI |
| Indication Studied: | Hemophilia B |
| Developmental Phase of Trial: | III |
| Name of the Sponsor/Company: | CSL Behring LLC<br>1020 First Avenue<br>King of Prussia<br>Pennsylvania 19406, USA |
| Name of the Monitoring CRO: | Medpace (with oversight by uniQure biopharma B.V.) |
| Coordinating Investigator: | PPD |
| Protocol Version and Date: | Version 8.0 (Amendment 7.0) 07 Feb 2022<br>Version 7.0 (Amendment 6.0) 28 Jun 2021<br>Version 6.0 (Amendment 5.0) 04 Feb 2021<br>Version 5.0 (Amendment 4.0) 15 Oct 2020<br>Version 4.0 (Amendment 3.0) 30 Aug 2019<br>Version 3.0 (Amendment 2.0) 07 Dec 2018<br>Version 2.0 (Amendment 1.0) 02 Apr 2018<br>Version 1.0 (Original) 16 Feb 2018 |

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<br>PPD, Clinical Development | |

# INVESTIGATOR'S ACKNOWLEDGEMENT

I have read this

**Protocol ID**: CT-AMT-061-02

**Title:** Phase III, open-label, single-dose, multi-center multinational trial investigating a 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.

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 termination of my participation as an Investigator for this trial.

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:<br>(please handwrite, print or type) | |

Signature: _____ Date: _____

# SUMMARY OF CHANGES (PROTOCOL HISTORY)

| **Current Protocol Amendment** | | |
|---|---|---|
| **Summary of Change(s) Since Last Version of Approved Protocol:** See Section 13.2 | | |
| **Amendment Number:**<br>7.0 (Version 8.0) | **Amendment Date:**<br>07 Feb 2022 | **Global/Country/Site-Specific:**<br>Global |

| **List of All Previous Amendments** | | |
|---|---|---|
| **Summary of Change(s) for Previous Amendments:** See Section 13.3 | | |
| **Amendment Number** | **Date of Amendment** | **Global/Country/Site-Specific** |
| 6.0 (Version 7.0) | 28 Jun 2021 | Global |
| 5.0 (Version 6.0) | 04 Feb 2021 | Global |
| 4.0 (Version 5.0) | 15 Oct 2020 | Global |
| 3.0 (Version 4.0) | 30 Aug 2019 | Global |
| 2.0 (Version 3.0) | 07 Dec 2018 | Global |
| 1.0 (Version 2.0) | 02 Apr 2018 | Global |
| 0.0 (Version 1.0) | 16 Feb 2018 | Global |

| **List of All Addendum** | |
|---|---|
| **Date of Addendum** | **Global/Country/Site-Specific** |
| 27 Mar 2019 | Germany |
| 27 Feb 2019 | France |

# 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<br>AUS: PPD<br>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<br>PPD<br>PPD<br>PPD | PPD<br>PPD<br>PPD<br>PPD |

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# LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

## List of Abbreviations

| | |
|---|---|
| AAV | adeno-associated virus |
| 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) |
| ABR | annualized bleeding rate |
| ADaM | Analysis Data Model |
| ADR | adverse drug reaction |
| AE | adverse event |
| AFP | Alpha-fetoprotein |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase |
| aPTT | activated partial thromboplastin time |
| AST | aspartate aminotransferase |
| ATMP | advanced therapy medicinal product |
| CCI | CCI |
| CAP | Controlled Attenuation Parameter |
| CDISC | Clinical Data Interchange Standards Consortium |
| cDNA | complementary deoxyribonucleic acid |
| CHMP | Committee for Medicinal Products for Human Use |
| $C_{max}$ | Maximum concentration |
| COVID-19 | Coronavirus Disease 2019 |
| CRA | clinical research associate |
| CRO | Contract Research Organization |
| CRP | c-reactive protein |
| CSR | Clinical Study Report |
| DILI | Drug Induced Liver Injury |
| DMC | Data Monitoring Committee |
| eCRF | electronic case report form |
| ELISA | enzyme-linked immunosorbent assay |
| EMA | European Medicines Agency |
| CCI | CCI |
| EU | European Union |
| FAS | Full Analysis Set |
| FDA | Food and Drug Administration |
| FIX | coagulation factor IX |
| GFP | green fluorescent protein |
| γGT | gamma-glutamyl transferase |
| gc | genome copies |

| | |
|---|---|
| GCP | Good Clinical Practice |
| GEE | generalized estimating equation |
| GTWP | Gene Therapy Working Party |
| CCI | CCI |
| HBsAg | hepatitis B surface antigen |
| HBV DNA | hepatitis B virus deoxyribonucleic acid |
| HCV RNA | hepatitis C virus ribonucleic acid |
| CCI | CCI |
| hFIX | human coagulation factor IX |
| HIPAA | Health Insurance Portability and Accountability Act |
| CCI | CCI |
| ICH | International Council for Harmonisation (previously International Conference on Harmonisation) |
| 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 |
| IWRS | Interactive Web Response System |
| J.A.D.E. | Joint Tissue Activity and Damage Exam |
| MCP-1 | monocyte chemotactic protein-1 |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MHRA | Medicines and Healthcare Products Regulatory Agency (UK) |
| MSKUS | Musculoskeletal Ultrasound |
| NAB | Neutralizing antibody |
| NHP | non-human primate |
| NIAAA | National Institute of Alcohol Abuse and Alcoholism |
| PBGD | porphobilinogen deaminase |
| PCR | polymerase chain reaction |
| PP | Per Protocol |
| CCI | CCI |
| PROBE | Patient Reported Outcomes, Burdens, and Experiences |
| CCI | |
| QP | Qualified Person |
| qPCR | quantitative (real-time) polymerase chain reaction |
| RM | repeated measures |

| | |
|---|---|
| SAE | serious adverse event |
| SAP | Statistical Analysis Plan |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2 |
| SD | standard deviation |
| SDTM | Study Data Tabulation Model |
| SOC | System Organ Class |
| SUSAR | suspected unexpected serious adverse reaction |
| TEAE | Treatment-emergent adverse event |
| UK | United Kingdom |
| ULN | Upper limit of normal |
| US/USA | United States of America |
| VAS | Visual Analogue Scale |
| WFH | World Federation of Haemophilia |
| CCI | CCI |

# CLINICAL TRIAL PROTOCOL SYNOPSIS

| **Protocol Number:** CT-AMT-061-02 | **Investigational Medicinal Product:** AMT-061 (AAV5-hFIXco-Padua; etranacogene dezaparvovec) |
|---|---|
| **Title of the Trial:** Phase III, open-label, single-dose, multi-center multinational trial investigating a 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:** At least 50 subjects. | |
| **Planned Number of Sites and Site Location(s):** It is planned to conduct this trial in approximately 50 sites in the United States of America (USA), the European Union (EU), and the United Kingdom (UK). | |
| **Coordinating Investigator** | PPD |
| **Trial Period (Planned):** First Subject Screening: Q2 2018 Last Subject Last Visit: Q2 2025 | **Clinical phase:** III |
| **Indication/Trial Population:** Male subjects with severe or moderately severe hemophilia B. | |
| **Objectives:** **Primary:** To demonstrate the non-inferiority of AMT-061 ($2 \times 10^{13}$ gc/kg) during the 52 weeks following establishment of stable factor IX expression (months 6 to 18) post-treatment (AMT-061) follow-up compared to standard of care continuous routine factor IX prophylaxis during the lead-in phase, as measured by the annualized bleeding rate (ABR). **Secondary:** To demonstrate additional efficacy and safety aspects of systemic administration of 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. Three subjects have been treated with $2 \times 10^{13}$ genome copies (gc)/kg AMT-061 in an ongoing dose confirmation Phase IIb trial (CT-AMT-061-01). The initial treatment phase has been completed in this study and an interim analysis based on a minimum of 6 weeks of post-treatment follow-up data for each subject has been performed. The interim analysis data set includes a combined 24 weeks of observation. All 3 subjects had been diagnosed with severe hemophilia B, with corresponding circulating factor IX activity levels <1% of normal. Following treatment with AMT-061, an increased level of factor IX activity was observed in all 3 subjects with mean ± standard deviation [SD] factor IX activity of 30.6 ± 6.97% (range: 23.9-37.8%) at Week 6 and 28.5 ± 6.42% (range 20.6 – 37.8%) across visits. Factor IX activity was measured using the activated partial thromboplastin time (aPTT) assay performed at a central laboratory. Two out of three subjects had previously screen-failed another gene therapy study due to pre-existing neutralizing antibodies (NABs) to a different AAV vector. All 3 subjects had evidence of pre-existing NAB activity against adeno-associated viral vector serotype 5 (AAV5). No bleeding episodes have been reported with an ABR of 0; in the year prior to screening, the number of treatment-requiring bleeding episodes ranged between 1 and 5. No subjects required any infusions of factor IX replacement therapy post-AMT-061 treatment. No deaths, serious adverse events (SAEs), or treatment-emergent adverse events (TEAEs) resulting in early discontinuation from the trial have been reported. There have been 13 TEAEs reported in 2 subjects, including 2 TEAEs assessed as related to study treatment; all TEAEs were mild in severity. One subject experienced a mild, asymptomatic and transient increase in liver enzyme levels (specifically elevated aspartate aminotransferase [AST] levels), which resolved without any additional treatment. No T cell response was observed in any of the 3 subjects. None of the subjects developed inhibitory antibodies to factor IX. Overall, the dose of $2 \times 10^{13}$ gc/kg AMT-061 | |

was shown to be safe and well tolerated and resulted in mean factor IX activity levels approximately 30% of normal circulating factor IX 6 weeks after dosing.

AMT-061 is a derivative of AMT-060, which is being studied in an ongoing Phase I/II clinical trial in male subjects with severe or moderately severe hemophilia B. Both AMT-060 and AMT-061 consist of an identical rAAV5 capsid. While AMT-060 contains the coding sequence for 'wild-type' hFIX, a two-nucleotide change has been introduced within the hFIX coding sequence in AMT-061, resulting in a single amino acid substitution, where Arginine is replaced by Leucine at position 338 (R338L) of the mature factor IX protein. The intended change in the mature protein represents the hFIX-Padua variant and it is responsible for the observed increased factor IX activity per unit of dose achieved with AMT-061 as compared to its predecessor AMT-060. The isolated modification leading to the representation of the hFIX-Padua variant in AMT-061 is not expected to influence other established safety characteristics of AAV5.

The results with AMT-061 to date are complemented by robust efficacy and safety data obtained with AMT-060 in the ongoing Phase I/II trial (CT-AMT-060-01) in ten subjects with hemophilia B. These ten subjects have now been followed for up to 2.5 years after treatment. The data demonstrate that subjects achieve clinically relevant and stable factor IX activity levels for up to 2.5 years after treatment with AMT-060. In addition, the AMT-060 treatment remains safe and well-tolerated. Alanine aminotransferase (ALT) increase was reported for 3 out of the 10 subjects in the study, however the ALT increases did not lead to loss of factor IX activity and no T-cell response was observed in these subjects. There was no loss of factor IX activity over time, and there were no immune responses and no development of factor IX inhibitors for any of the 10 subjects in the study.

The purpose of this Phase III trial is to demonstrate the efficacy of AMT-061 in terms of ABR, to further describe its efficacy in terms of endogenous factor IX activity, and to further describe its safety profile. The efficacy and safety results obtained during the Phase IIb study with AMT-061 (CT-AMT-061-01), supplemented with the strong efficacy and safety results obtained during the Phase I/II trial with AMT-060 (CT-AMT-060-01), demonstrate $2 \times 10^{13}$ gc/kg to be the optimal dose for use in this pivotal Phase III trial.

**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 intravenous (IV) infusion.

The single administered dose of AMT-061 will be $2 \times 10^{13}$ gc/kg.

**Reference Therapy:**

The reference therapy is the prophylaxis factor IX replacement therapy used during the lead-in phase, prior to treatment with AMT-061.

**Trial Design/Methodology:**

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

Screening (Visit S)

At the screening visit (Visit S), subjects are assessed for eligibility. Subjects will be asked to complete the CCI [redacted] and the CCI [redacted] will be assessed. Screening assessments will also include medical history, vital signs, physical examination, and blood sampling. A FibroScan™ will be performed with liver fibrosis scores collected during the assessment. If possible, steatosis (Controlled Attenuation Parameter) scores should also be collected during the assessment. If a site does not have access to FibroScan, a Sponsor-preapproved alternative may be performed. At this visit, subjects will receive their electronic diary (e-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 their dedicated e-diary. The period between the screening visit up to the start of the lead-in phase (Visit L1) 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 the Investigator/study nurse. From screening onwards, the Investigator or designee will have regular discussions with the subject regarding the importance of a

healthy liver before and after receiving a liver directed gene therapy, and factors that might impact liver health (including acetaminophen and alcohol intake).

Lead-in phase (Visit L1 to L-Final)

Eligible subjects will enter the lead-in phase. The length of the lead-in phase is a minimum of 6 months (26 weeks), ending at or before Visit D. Subjects will remain in the lead-in phase until a minimum of 6 months of lead-in data have been collected and it is confirmed that the subjects still meet all eligibility criteria, with the final visit in this phase (Visit L-Final) occurring approximately four weeks (6 weeks maximum) prior to the planned date of IMP dose administration (Visit D).

Prior to the start of the lead-in phase (Visit L1), subjects will undergo a wash out period from their usual factor IX product/dose. The wash out period will be 3 days for regular-acting factor IX products and 10 days for extended half-life factor IX products. At Visit L1, blood samples for assessment of factor IX activity and factor IX protein concentration will be taken.

During the lead-in phase, subjects will record their use of prophylactic factor IX replacement therapy and bleeding episodes in their e-diary. e-diary data will be reviewed on a continuous basis by the Investigator/study nurse, with alternating monthly clinic visits and follow-up phone calls (in line with the trial schedule and with retraining by phone as needed). In addition to the subjects' reporting of bleeding episodes in the e-diary, the Investigator or designee will assess each bleeding episode 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 bleeding, the subject must be called and/or visit the site. At the clinic visits during this lead-in phase (occurring every two months), blood samples will be taken for assessment of factor IX activity, transaminases, and AAV5 antibodies. Subjects will be asked to complete the CCI [redacted] at Visit L3.

If the informed consent form (ICF) is revised during the lead-in phase with important new information that must be shared with the study subjects, the amended ICF will be presented, as required by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), for review and consideration by the subject, and signed re-consent is to be obtained prior to IMP dose administration. During Visit L-Final, the subject's eligibility will be re-evaluated based on assessments during the lead-in period and at Visit L-Final. Blood samples will be taken and the laboratory values obtained at this visit will be used to determine eligibility for dosing and continued participation in the trial. At this visit, subjects will be asked to complete the CCI [redacted], CCI [redacted] will be assessed, a physical examination will be performed, and vital signs will be measured. The baseline abdominal ultrasound should be done at Visit L-Final at the latest. Additionally, blood samples for the factor IX recovery assessment will be taken before and 30 minutes after (timed from end of infusion) subjects have taken their usual factor IX treatment at this visit.

IMP dose administration (treatment; Visit D) + Post-treatment follow-up phase (Visit F1 to F-Final [Week 1 to Week 52/Month 12])

At Visit D, pre-IMP dose administration, subject's eligibility will be confirmed, vital sign measurements will be taken, a physical examination will be performed, and blood samples will be taken for assessment of factor IX activity, routine laboratory parameters, transaminases, and AAV5 antibodies.

Once eligibility is confirmed and all baseline assessments have been performed, subjects will receive a single infusion of AMT-061. After IMP administration (post-IMP), subjects will be monitored at the clinical trial site for 3 hours for tolerance to the IMP and detection of potential immediate adverse events (AEs).

Subjects will then be followed for one year (52 weeks) in the post-treatment follow-up phase (Visit F1 to F-Final [Week 1 to Week 52/Month 12]). Subjects will have weekly visits for the first 12 weeks (Visit F1 to F12 [Week 1 to 12]), and monthly visits from Month 4 to Month 12 (Visit F13 to F-Final [Month 4 to Month 12/Week 52]). With agreement from the Investigator and Sponsor, visits that do not include physical examination and/or CCI [redacted] may be performed at the subject's home by an appropriately qualified and trained nurse. Each home nursing visit is expected to be supplemented by a phone call from the Investigator or designee to the subject to discuss AEs, concomitant medications, and e-diary compliance. 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.

Subjects are permitted to continue their continuous routine factor IX treatment in the first weeks after dosing. During the post-treatment follow-up visits, endogenous factor IX activity will be assessed. If the endogenous factor IX activity is ≥5%, continuous routine factor IX prophylaxis will be discontinued and further management will be based on Investigator's clinical judgement and subject preference. Continuation or re-initiation of continuous routine factor IX 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 judgement and subject preference. If endogenous factor IX activity is <2%, continuous routine factor IX prophylaxis must be continued or reinstated. Additional on-demand and/or intermittent prophylactic factor IX treatment may be given after treatment with AMT-061, if considered necessary.

During the post-treatment follow-up phase, subjects will continue to record their use of factor IX replacement therapy and bleeding episodes in the e-diary, following the same principles as during the lead-in phase. Further samples will be taken for safety and efficacy laboratory parameters. Subjects will complete the CCI at Visit F15 (Month 6) and Visit F-Final (Month 12), and CCI will be assessed at Visit F-Final. Additionally, an abdominal ultrasound will be performed at Visit F-Final. Use of concomitant medication and occurrence of AEs will be continuously monitored. The discussions with the subject on the importance of a healthy liver, and factors that might impact liver health (including acetaminophen and alcohol intake), will continue.

For ALT level increments of at least 2-fold baseline (i.e., Visit D, 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 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.

The first secondary endpoint, endogenous factor IX activity at 6 months after IMP administration, will be assessed once the last subject has achieved 6 months after AMT-061 treatment. The second secondary endpoint, endogenous factor IX activity at 12 months after IMP administration, will be assessed once the last subject has achieved 12 months after AMT-061 treatment. After 52 weeks following stable factor IX expression, all available efficacy and safety data collected between screening and 18 months post-treatment will be analyzed and reported in a Clinical Study Report (CSR), including (but not limited to), the primary ABR endpoint, and the previously assessed secondary endpoints related to factor IX activity at 6 and 12 months.

Long-term follow-up phase (Visit LTF1 to LTF8 [Month 18 to Month 60])

After the post-treatment follow-up phase, 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 who are on continuous routine factor IX prophylaxis during the long-term follow-up phase of the trial are required to contact the site staff immediately in case of a bleed and/or factor IX use different from their routine factor IX prophylaxis, in addition to completing the questions/information requested on the paper diaries to capture all information.

Subjects visit the clinic every half year (6 months) for evaluation of efficacy parameters and safety. Every 6 months an abdominal ultrasound will be performed. Every year (12 months), subjects will complete the CCI and CCI will be assessed. Occurrence of AEs will be continuously monitored, with at least quarterly contact between the site staff and subject; proper completion of the study-specific paper diaries and proper reporting of factor IX usage and bleeding episodes will also be monitored at these quarterly contacts. Adjustments to the visit location or schedule may be made to accommodate safety concerns and restrictions due to the 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.

At the end of the trial, all safety and efficacy data from the long-term follow-up will be analyzed and reported in a CSR addendum.

Please refer to Figure 1 and Figure 2 for an overview of the study and its design and to Table 1 through Table 4 for an overview of the assessments and testing performed throughout these different trial phases.

Optional Patient Reported Outcomes, Burdens, and Experiences (PROBE) Questionnaire Sub-study

Subjects who consent to this sub-study will complete the PROBE questionnaire at the same visits as the other CCI [redacted]. Adjustments to the visit schedule may be made to accommodate safety concerns and restrictions due to the COVID-19 pandemic.

Optional Musculoskeletal Ultrasound (MSKUS) Sub-study

Subjects who consent to this optional sub-study will undergo musculoskeletal ultrasounds (MSKUS) at screening (Visit S), Visit L-Final, during the post-treatment follow-up at Visits F15 (Month 6) and F-Final (Month 12/Week 52), and during the long-term follow-up at Visits LTF2, LTF4, LTF6, and LTF8 (Month 24, 36, 48, and 60, respectively). Adjustments to the visit schedule may be made to accommodate safety concerns and restrictions due to the COVID-19 pandemic.

**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 joint health, and the CCI [redacted] on general and disease specific CCI [redacted]. The PROBE questionnaire and MSKUS will be assessed for subjects participating in these sub-studies.

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 with known severe or moderately severe factor IX deficiency (≤2% of normal circulating factor IX) for which the subject is on continuous routine factor IX prophylaxis*
4. >150 previous exposure days of treatment with factor IX protein
5. Have been on stable prophylaxis for at least 2 months prior to screening
6. Have demonstrated capability to independently, accurately and in a timely manner complete the diary during the lead-in phase as judged by the Investigator
7. 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)
8. 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 and Visit L-Final (based on local laboratory results)
3. Screening and Visit L-Final laboratory values (based on central laboratory results):
   a. ALT >2 times ULN
   b. AST >2 times ULN
   c. Total bilirubin >2 times ULN (except if this is caused by Gilbert disease)
   d. Alkaline phosphatase (ALP) >2 times ULN
   e. Creatinine >2 times ULN
4. Positive human immunodeficiency virus (HIV) serological test at screening and Visit L-Final, not controlled with anti-viral therapy as shown by CD4+ counts ≤200/µL (based on central laboratory results)
5. Hepatitis B or C infection with the following criteria present at screening:
   a. Currently receiving antiviral therapy for this/these infection(s)
      and/or
   b. Positive for any of the following (based on central laboratory results):

i. Hepatitis B surface antigen (HBsAg), except if in the opinion of the Investigator this is due to a previous Hepatitis B vaccination rather than active Hepatitis B infection
ii. Hepatitis B virus deoxyribonucleic acid (HBV DNA)
iii. Hepatitis C virus ribonucleic acid (HCV RNA)

6. Known coagulation disorder other than hemophilia B
7. Thrombocytopenia, defined as a platelet count below $50 \times 10^9$/L, at screening and Visit L-Final (based on central laboratory results)
8. 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
9. Known significant medical condition that may significantly impact the intended transduction of the vector and/or expression and activity of the protein, including but not limited to:
   a. Disseminated intravascular coagulation
   b. Accelerated fibrinolysis
   c. Advanced liver fibrosis (suggestive of or equal to METAVIR Stage 3 disease; e.g., a FibroScan™ score of ≥9 kPa is considered equivalent)
10. Known history of an allergic reaction or anaphylaxis to factor IX products
11. Known history of allergy to corticosteroids
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.

**Planned Duration of Subject Involvement in the Trial:**

- Planned duration of screening/enrolment period: approximately 4 weeks
- Planned duration of lead-in phase: at least 26 weeks (6 months)
- 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 and Statistical Analysis:**

Primary efficacy endpoints

- ABR comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment)

Secondary efficacy endpoints

- Endogenous factor IX activity at 6 months after AMT-061 dosing
- Endogenous factor IX activity at 12 months after AMT-061 dosing
- Endogenous factor IX activity at 18 months after AMT-061 dosing
- Annualized consumption of factor IX replacement therapy during the 52 weeks following stable factor IX expression (months 6-18 post-treatment), excluding factor IX replacement for invasive procedures, compared to the lead-in phase
- Annualized infusion rate of factor IX replacement therapy during the 52 weeks following stable factor IX expression (months 6-18 post-treatment), excluding factor IX replacement for invasive procedures, compared to the lead-in phase
- Proportion of subjects remaining free of previous continuous routine prophylaxis during the 52 weeks following stable factor IX expression (months 6-18 post-treatment)
- Comparison of the percentage of subjects with trough factor IX activity <12% of normal between the lead-in phase and after treatment with AMT-061 over the 52 weeks following stable factor IX expression (months 6-18 post-treatment)

- ABR comparison between AMT-061 and prophylaxis for superiority between the lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment)
- Rate of spontaneous bleeding events during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) compared to the lead-in phase
- Rate of joint bleeding events during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) compared to the lead-in phase
- Estimated ABR – during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) – as a function of pre-IMP anti-AAV5 antibody titers using the luciferase based NAB assay (as a "correlation" analysis)
- Correlation of factor IX activity levels during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) with pre-IMP anti-AAV5 antibody titers using the luciferase based NAB assay
- Occurrence of (and resolution of) new target joints during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) and resolution of pre-existing target joints following AMT-061 dosing
- Proportion of subjects with zero bleeds during the 52 weeks following stable factor IX expression (months 6-18 post-treatment)
- CCI [redacted] during the 12 months following AMT-061 dosing compared with the lead-in phase
- CCI [redacted] score during the 12 months following AMT-061 dosing compared with the lead-in phase

CCI [redacted]

CCI

Sub-study endpoints

- PROBE summary scores and individual item responses.
- MSKUS sub-study endpoints
  - Joint Tissue Activity and Damage Exam (J.A.D.E.) scores

Secondary safety endpoints

- Adverse events (AEs)
- Changes in abdominal ultrasound
- Anti-AAV5 antibodies (total [IgM and IgG], NABs)
- AAV5 capsid-specific T cells
- Anti-factor IX antibodies
- Factor IX inhibitors and recovery
- Hematology and serum chemistry parameters
- ALT/AST levels, and corticosteroid use for ALT/AST increases
- Vector DNA in blood and semen
- Inflammatory markers: IL-1β, IL-2, IL-6, IFNγ, MCP-1
- Alpha-fetoprotein (AFP)

Analysis Populations

The Full Analysis Set (FAS) will include all subjects who are enrolled, entered the lead-in phase, were dosed with AMT-061, and provide at least one efficacy endpoint assessment subsequent to AMT-061 dosing. The FAS population will be the primary population for all efficacy statistical analyses.

The PP population will include all subjects from the FAS population who adhere to a stable and adequate prophylaxis use during the lead-in phase, who complete at least 18 months of efficacy assessments (52 weeks after achieving stable factor IX expression) for the 18-month (data cut) analysis, who complete at least a full year of efficacy assessments for the 12-month (data cut) analysis, or who complete at least 6 months of efficacy assessments for the 6-month (data cut) analysis, and who have no major protocol deviations that impact the interpretation of efficacy. The PP population will be used for sensitivity analyses.

The lead-in safety population will consist of all subjects who receive lead-in treatment (i.e. who are enrolled into the lead-in period). The post-treatment safety population will consist of all subjects who receive AMT-061, irrespective of any protocol deviations. The safety population will consist of all subjects who are in either the lead-in safety population or the post-treatment safety population.

Sample Size

The study sample size is constrained by the non-inferiority analysis of the primary endpoint, ABR. Via simulation of ABR under a negative binomial distribution with a yearly rate of 2.4 events for lead-in and 1.9 for post-treatment, with a Pearson correlation of 0.05 for the number of events between the two periods, and with a common negative binomial dispersion parameter of 1.5, a sample size of N=50 will demonstrate non-inferiority with a non-inferiority margin of 1.8 and a power of 82.0%. Therefore, the study should consist of at least 50 analyzable subjects.

Statistical Methods

*Primary efficacy analyses*

The primary aim of the trial is to demonstrate the non-inferiority of AMT-061 ($2 \times 10^{13}$ gc/kg) during the 52 weeks following establishment of stable factor IX expression (months 6 to 18) post-treatment (AMT-061) follow-up compared to standard of care continuous routine factor IX prophylaxis during the lead-in phase, as measured by the ABR.

Single treatment with AMT-061 will be claimed to be non-inferior to factor IX prophylaxis treatment when the upper limit of the one-sided 97.5% confidence interval of the rate ratio in ABR between AMT-061 (post-treatment) and factor IX prophylaxis (lead-in) is less than 1.8.

ABR will be determined for the lead-in period and post-treatment period (for the 52 weeks following stable factor IX expression [months 6-18 post-treatment with AMT-061]). Analysis of the number of reported bleeding events will be performed using a repeated measures (RM) generalized estimating equation (GEE) negative binomial regression model accounting for the paired design of the trial with an offset parameter to account for the differential collection periods. The estimated rate ratio, one-sided 97.5% Wald confidence interval, and corresponding p-value will be determined. The upper limit of the resultant confidence interval of the rate ratio will be compared to the non-inferiority margin of 1.8. If the upper limit is less than 1.8, then non-inferiority will be declared.

The primary efficacy analysis of ABR for the non-inferiority assessment will be completed using the FAS.

*Order of testing of primary and secondary endpoints*

Formal statistical testing of the efficacy endpoints will be performed using the closed testing principle (for Type I error control for multiple testing). Due to the closed testing principle, no correction for multiplicity is necessary. All endpoints will be tested for superiority at a one-sided alpha level of 0.025 (except as otherwise noted). Superiority and non-inferiority testing will be accomplished using the FAS population. Fixed sequential testing will be performed using a hierarchical approach and will be continued until a non-significant result is obtained (except as otherwise noted). The primary and secondary endpoints will be analyzed in the following order.

1. ABR comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment; primary efficacy endpoint)

2. Endogenous factor IX activity at 6 months after AMT-061 (first secondary efficacy endpoint)
3. Endogenous factor IX activity at 12 months after AMT-061 (second secondary efficacy endpoint)
4. Endogenous factor IX activity at 18 months after AMT-061 (third secondary efficacy endpoint)
5. Annualized consumption of factor IX replacement therapy during the 52 weeks following stable factor IX expression (months 6-18 post-treatment), excluding factor IX replacement for invasive procedures, compared to the lead-in phase (secondary efficacy endpoint)
6. Annualized infusion rate of factor IX replacement therapy during the 52 weeks following stable factor IX expression (months 6-18 post-treatment), excluding factor IX replacement for invasive procedures, compared to the lead-in phase (secondary efficacy endpoint)
7. Comparison of the percentage of subjects with trough factor IX activity <12% of normal between the lead-in phase and after treatment with AMT-061 over the 52 weeks following stable factor IX expression (months 6-18 post-treatment; secondary efficacy endpoint)
8. ABR comparison between AMT-061 and prophylaxis for superiority between the lead-in and the 52 weeks following stable factor IX expression (months 6-18 post-treatment); secondary efficacy endpoint)
9. Rate of spontaneous bleeding events during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) compared to the lead-in phase (secondary efficacy endpoint)
10. Rate of joint bleeding events during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) compared to the lead-in phase (secondary efficacy endpoint)
11. CCI [redacted] during the 12 months following AMT-061 dosing compared with the lead-in phase (secondary efficacy endpoint)
12. CCI [redacted] during the 12 months following AMT-061 dosing compared with the lead-in phase (secondary efficacy endpoint)

CCI [redacted]

[redacted]

*Safety analyses*

Adverse events will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA). Total treatment-emergent AEs (TEAEs), and broken down by relationship, severity, seriousness, will be presented descriptively by system organ class and preferred term, presenting number and percentage of subjects and number of events.

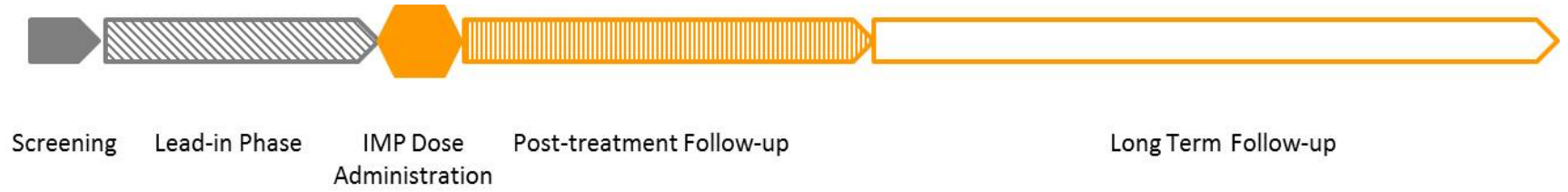
All other safety data will be presented using graphical displays, descriptive statistics (including change from baseline, if applicable), and/or individual data listings.

The number of days until vector DNA can no longer be detected in blood and semen will be tabulated. The number of days is calculated using the date of collection of the first of three consecutive sample negative for vector DNA for each matrix.

All safety analyses will be based on the safety population.

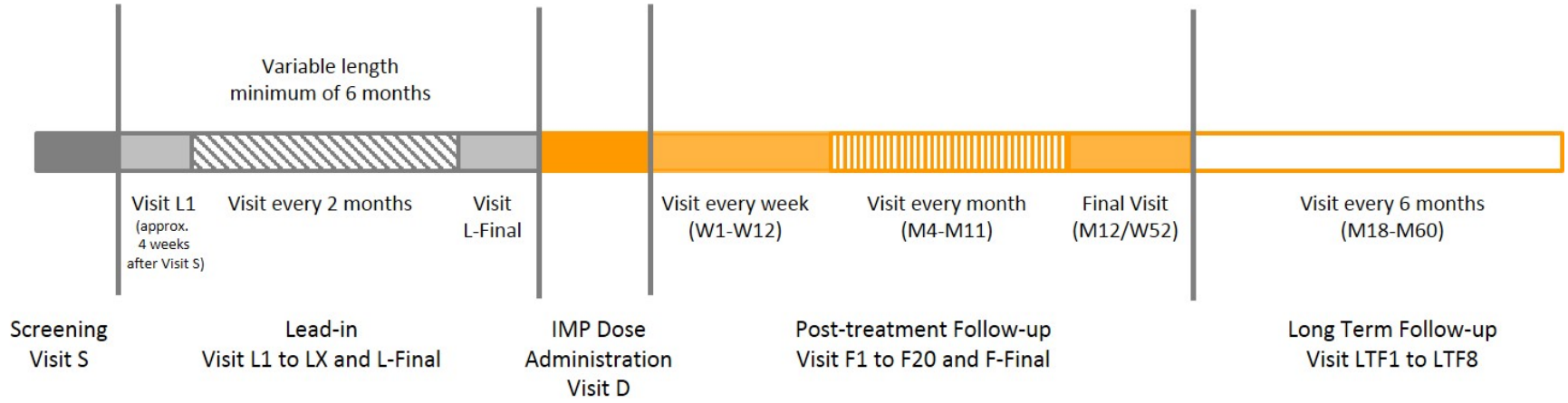
Analyses will be based on central laboratory measurements if results are available from both local and central laboratories.

## Study Design Schema




Abbreviations: IMP = Investigational Medicinal Product

**Figure 1 Study Overview**




Abbreviations: IMP = Investigational Medicinal Product

**Figure 2 Study Design**

## Trial Schedules

**Table 1 Schedule of Events for Efficacy and Safety Evaluation, for Screening (Visit S), Lead-in (Visit L1 to L-Final) and Treatment (Visit D) + Post-Treatment Follow-up (Visit F1 to F-Final)**

| **Trial Period** [a] | **Screening** | **Lead-in** [b, c, d] | | | **IMP Dose Administration** [d] | | | **Post-treatment Follow-up** [d, e, f] | | | **Additional Visits** [g] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day / Week / Month** | Visit S [h] | Visit L1 (L-W0)[i] | Visit L2 to LX[j] (every 2 months, starting at L-M2) | Visit L-Final | Visit D pre-IMP | Visit D 0h | Visit D post-IMP 3h | Visit F1 to F12 (every week W1 to W12) | Visit F13 to F20 (every month M4 to M11) | Visit F-Final (M12/W52) | |
| **Visit window (days)** | -28 | 0 | ±14 | [j] | 0 | 0 | ±15 minutes | ±2 | ±5 | ±5 | |
| Informed consent | x | | | | | | | | | | |
| Inclusion/exclusion criteria | x | | | x | x [k] | | | | | | |
| Medical history | x | | | | | | | | | | |
| Administration of IMP | | | | | | x | | | | | |
| Hand-out of subject treatment card [l] | | | | | | | x | | | | |
| Concomitant medication | Continuous | | | | | | | | | | (x) [g] |
| Vital signs (blood pressure, pulse, body temperature) | x | | | x | x | | x | x | x | x | (x) [g] |
| Physical examination | x | | | x | x | | | x: Visit F1, F2, F4, F6, F12 (W1, 2, 4, 6, 12) | x: Visit F13, F15, F17, F19 (M4, 6, 8, 10) | x | (x) [g] |
| Height | x | | | | | | | | | | |
| Body weight | x | | | x | | | | | | | (x) [g] |
| FibroScan™ [m] | x | | | | | | | | | | |
| Blood and semen sampling (see Table 2) | x | x | x | x | x | | x | x | x | x | (x) [g] |
| Subject provided e-diary device and e-diary instruction [n] | x | | | | | | | | | | |
| Recording of e-diary data by subject (bleeding episodes, factor IX replacement therapy) | Continuous | | | | | | | | | | |

| **Trial Period** [a] | **Screening** | **Lead-in** [b, c, d] | | | **IMP Dose Administration** [d] | | | **Post-treatment Follow-up** [d, e, f] | | | **Additional Visits** [g] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day / Week / Month** | Visit S [h] | Visit L1 (L-W0)[i] | Visit L2 to LX[j] (every 2 months, starting at L-M2) | Visit L-Final | Visit D pre-IMP | Visit D 0h | Visit D post-IMP 3h | Visit F1 to F12 (every week W1 to W12) | Visit F13 to F20 (every month M4 to M11) | Visit F-Final (M12/W52) | |
| **Visit window (days)** | -28 | 0 | ±14 | [j] | 0 | 0 | ±15 minutes | ±2 | ±5 | ±5 | |
| Review of e-diary data by Investigator/study nurse | Continuous | | | | | | | | | | (x) [g] |
| Collection of e-diary device | | | | | | | | | | x | |
| Subject provided with paper diaries and paper diary instruction for Long-Term Follow-up Phase | | | | | | | | | | x | |
| Assessment of bleeding episode(s) by Investigator [o] | Continuous | | | | | | | | | | (x) [g] |
| Liver health discussion (including acetaminophen and alcohol intake) [p] | Continuous | | | | | | | | | | |
| Abdominal ultrasound [q] | | | | x | | | | | | x | |
| CCI | x | | x: Visit L3 | x | | | | | x: Visit F15 (M6) | x | |
| CCI | x | | x: Visit L3 | x | | | | | x: Visit F15 (M6) | x | |
| CCI | x | | x: Visit L3 | x | | | | | x: Visit F15 (M6) | x | |
| CCI | x | | x: Visit L3 | x | | | | | x: Visit F15 (M6) | x | |
| CCI | x | | x: Visit L3 | x | | | | | x: Visit F15 (M6) | x | |
| CCI | x | | x: Visit L3 | x | | | | | x: Visit F15 (M6) | x | |
| CCI | x | | | x | | | | | | x | |
| PROBE [r, s] | x | | x: Visit L3 | x | | | | | x: Visit F15 (M6) | x | |
| Musculoskeletal ultrasound [t] | x | | | x | | | | | x: Visit F15 (M6) | x | |
| Adverse events | Continuous | | | | | | | | | | (x) [h] |

CCI; D: dosing; h: hour; CCI; CCI; CCI; IMP: investigational medicinal product; CCI; L-M: lead-in month; L-W: lead-in week; M: month; PROBE = Patient Reported Outcomes, Burdens, and Experiences; CCI; W: week; CCI

a. This table shows events for screening, lead-in, and treatment + post-treatment follow-up. Refer to Table 3 for events during the long-term follow-up.
b. Each visit in the lead-in phase is scheduled in relation to the date of L1 (L-W0), not in relation to the date of its previous visit.
c. The lead-in phase will be a minimum of 6 months, ending at or before Visit D. Visits will occur every 2 months and between visits, there will be a follow-up telephone call for e-diary review (alternating months).
d. For subjects on continuous 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 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 continuous 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 5 half-lives of exogenous Factor IX product use. In case the 5 half-lives washout extends beyond the protocol allowed visit window, the Medpace and uniQure Medical Directors are to be contacted to discuss how to proceed.
e. Each visit in the post-treatment follow-up is scheduled in relation to the date of IMP dose administration (Visit D), not in relation to the date of its previous visit.
f. With agreement from the Investigator and Sponsor during the Post-Treatment Follow-Up period (from Visit D up until Week 52), visits that do not include physical examination and/or CCI may be performed at the subject's home by an appropriately qualified and trained nurse. Each home nursing visit is expected to be supplemented by a phone call from the Investigator or designee to the subject to discuss AEs, concomitant medications, and e-diary compliance.
g. An additional visit can be performed at any time during the study (lead-in or follow-up phases) for the purpose of conducting one or more procedures listed in the column "Additional Visits", as per the preference of the Investigator. Hence, "(x)" refers to a procedure that can be performed, if judged relevant by the Investigator.
h. The approximately 4-week period between the screening visit (Visit S) and the start of the lead-in phase (Visit L1) 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. With prior approval from the Sponsor, screening procedures can occur during the duration of the screening period.
i. Prior to the Visit L1, subjects will undergo a wash out period from their usual factor IX product/dose. The wash out period will be 3 days for regular-acting factor IX products and 10 days for extended half-life factor IX products.
j. Visit window for Visit L-Final is -28 days (±7 days) from Visit D. The time period between Visit LX and Visit L-Final can be less than 2 months as long as the total lead-in period is a minimum of 26 weeks.
k. Laboratory assessments from Visit L-Final will be used for inclusion/exclusion criteria at Visit D.
l. When all assessments at 3 hours post-IMP administration are performed, the subject treatment card will be handed out and the subject may leave the clinic. If a subject is traveling to a different site for Visit D, this card should be handed out at the L-Final visit and the subject should be instructed to bring it with him to Visit D.
m. Liver fibrosis scores will be collected during the FibroScan assessment. If possible, steatosis (Controlled Attenuation Parameter [CAP]) scores should also be collected. If a site does not have access to FibroScan, a Sponsor-preapproved alternative may be performed. The FibroScan should preferably be done at the screening visit or at an alternative time point, however at L-Final at the latest. If a FibroScan assessment has been performed within the year prior to screening for which CAP scores are available or if a liver biopsy has been performed within the 2 years prior to the screening visit and fibrosis grade was documented, a FibroScan is not necessary.
n. Instruction can be repeated at any visit as deemed necessary by the Investigator or study nurse.
o. The Investigator or designee will assess each bleeding episode as soon as possible, but at least within 72 hours after it has been reported by the subject.
p. From screening onwards, the Investigator or designee will have regular discussions with the subject regarding the importance of a healthy liver before and after receiving a liver directed gene therapy, and factors that might impact liver health (including acetaminophen and alcohol intake). It is recommended that acetaminophen total daily dose per subject be limited to ≤2 g/day. It is also recommended that subjects on the study should not consume >20 g of alcohol per day. While it is recommended that the daily alcohol limit in grams be adhered to most strictly during the first 12 weeks post-IMP, the importance of abstaining from binge-drinking at any point post IMP administration should be reinforced at every visit.
q. The baseline abdominal ultrasound should be done at L-Final at the latest. For those subjects where Visit F-Final (Month 12) is 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.
r. It is recommended that the CCI (CCI/PROBE]) 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 if possible, 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 recommended to be 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 Visit F15 and Visit F-Final 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.

s. Applicable only for subjects participating in the optional PROBE questionnaire sub-study. For those subjects where the Visit F15 and Visit F-Final are impacted by COVID-19, the PROBE questionnaire 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.

t. Applicable only for subjects participating in the optional musculoskeletal ultrasound (MSKUS) sub-study. If it is not possible to obtain the MSKUS at screening (Visit S), it is allowed to obtain this first MSKUS at a later time point (preferably as soon as possible thereafter during one of the lead-in visits). For those subjects where the Visit F15 and Visit F-Final are impacted by COVID-19, scans may be conducted 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 MUSKUS. Adjustments to the visit timing within this window are to be documented.

**Table 2 Schedule of Events for Laboratory Parameters, for Screening (Visit S), Lead-in (Visit L1 to L-Final) and Treatment + Post-Treatment Follow-up (Visit F1 to F-Final)**

| Trial Period [a] | Screening | Lead-in [b, c, d] | | | IMP Dose Administration [d] | | | Post-treatment Follow-up [d, e] | | | Additional Visits [f] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day / Week / Month** | Visit S | Visit L1 (L-W0)[g] | Visit L2 to LX (every 2 months, starting at L-M2) | Visit L-Final | Visit D pre-IMP | Visit D 0h | Visit D post-IMP 3h | Visit F1 to F12 (every week W1 to W12) | Visit F13 to F20 (every month M4 to M11) | Visit F-Final (M12/W52) | |
| **Visit window (days)** | -28 | 0 | ±14 | [h] | 0 | 0 | ±15 minutes | ±2 | ±5 | ±5 | |
| **Local Laboratory** | | | | | | | | | | | |
| Factor IX: one-stage aPTT for factor IX activity for local monitoring | x | x | x | x | x | | | x | x | x | (x) [f] |
| Factor IX inhibitors (Bethesda assay or Nijmegen modified Bethesda assay) for local monitoring and eligibility check [i] | x | | | x | x | | | x: Visit F6, F12 (W6, W12) | x: Visit F15 (M6) | x | (x) [f] |
| Transaminases (AST/ALT) for local monitoring [j] | x | | x | x | x | | | x | x | x | (x) [f] |
| **Central Laboratory** | | | | | | | | | | | |
| One-stage aPTT for factor IX activity | | x | x | x | x | | | x | x | x | (x) [f] |
| Chromogenic Assay for factor IX activity | | x | x | x | x | | | x | x | x | (x) [f] |
| Factor IX protein concentration | | x | | x | x | | | x | x | x | (x) [f] |
| Anti-factor IX antibodies | | | | | x | | | x: Visit F6, F12 (W6, W12) | x: Visit F15 (M6) | x | (x) [f] |
| Factor IX inhibitors (Nijmegen modified Bethesda assay) [i] | x | | | x | x | | | x: Visit F6, F12 (W6, W12) | x: Visit F15 (M6) | x | (x) [f] |
| Factor IX recovery [k] | | | | x | | | | | | | (x) [f] |
| Total (IgM and IgG) antibodies to AAV5 | x | | x | x | x | | | x: Visit F3, F6, F9, F12 (W3, W6, W9, W12) | x: Visit F15 (M6) | x | (x) [f] |
| Neutralizing antibodies to AAV5 | x | | x | x | x | | | x: Visit F3, F6, F9, F12 (W3, W6, W9, W12) | x: Visit F15 (M6) | x | (x) [f] |
| AAV5 capsid-specific T cells | | | | | x | | | x | x | x | (x) [f] |

| Trial Period [a] | Screening | Lead-in [b, c, d] | | | IMP Dose Administration [d] | | | Post-treatment Follow-up [d, e] | | | Additional Visits [f] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day / Week / Month** | Visit S | Visit L1 (L-W0)[g] | Visit L2 to LX (every 2 months, starting at L-M2) | Visit L-Final | Visit D pre-IMP | Visit D 0h | Visit D post-IMP 3h | Visit F1 to F12 (every week W1 to W12) | Visit F13 to F20 (every month M4 to M11) | Visit F-Final (M12/W52) | |
| **Visit window (days)** | -28 | 0 | ±14 | [h] | 0 | 0 | ±15 minutes | ±2 | ±5 | ±5 | |
| Sampling for vector genome detection | | | | | | | | | | | |
| Blood [l] | | | | | x | | x | x | x | x | (x) [f] |
| Semen [l] | | | | | x[m] | | | x: Visit F6, F12 (W6, W12) | x: Visit F13, F15 (M4, M6) | x | (x) [f] |
| Inflammatory markers IL-1β, IL-2, IL-6, IFNγ, MCP-1 | | | | | x | | | x | x | x | (x) [f] |
| Alpha-fetoprotein (AFP) | | | | x | | | | | x: Visit F15 (M6) | x | (x) [f] |
| Hematology and coagulation parameters [n] | x | | | x | x | | | x | x | x | (x) [f] |
| Serum chemistry parameters [o] | x | | | x | x | | x (only CRP) | x | x | x | (x) [f] |
| HIV, viral load, and CD4+ | x | | | x | | | | | | | |
| HBsAg, HBV DNA and HCV RNA | x | | | x | | | | | | | |
| Factor IX gene sequencing [p] | x | | | | | | | | | | |
| Blood sample for future research [q] | x | | | | x | | | x: Visit F12 (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; HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; 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; M: month; MCP-1: monocyte chemotactic protein-1; W: week.

a. This table shows events for screening, lead-in, and treatment + post-treatment follow-up. Refer to Table 4 for events during the long-term follow-up.
b. Each visit in the lead-in phase is scheduled in relation to the date of L1 (L-W0), not in relation to the date of its previous visit.
c. The lead-in phase will be a minimum of 6 months, ending at or before Visit D. Visits will occur every 2 months and between visits, there will be a follow-up telephone call for e-diary review (alternating months).
d. For subjects on continuous 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 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 continuous 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 5 half-lives of exogenous Factor IX product use. In case the 5 half-lives washout extends beyond the protocol allowed visit window, the Medpace and uniQure Medical Directors are to be contacted to discuss how to proceed.

e. Each visit in the post-treatment follow-up is scheduled in relation to the date of IMP dose administration (Visit D), not in relation to the date of its previous visit.
f. An additional visit can be performed at any time during the study (lead-in or follow-up phases) for the purpose of conducting one or more procedures listed in the column "Additional Visits", as per the preference of the Investigator. Hence, "(x)" refers to a procedure that can be performed, if judged relevant by the Investigator.
g. Prior to the Visit L1, subjects will undergo a wash out period from their usual factor IX product/dose. The wash out period will be 3 days for regular-acting factor IX products and 10 days for extended half-life factor IX products.
h. Visit window for Visit L-Final is -28 days (±7 days) from Visit D.
i. In case of a positive test for factor IX inhibitors, a re-test should be performed 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.
j. For ALT level increments of at least 2-fold baseline (i.e., Visit D, pre-IMP) and/or > upper limit of normal (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.
k. At suspicion of factor IX inhibitor, the factor IX recovery assessment should be repeated. Blood samples for factor IX recovery assessment should be drawn prior to administering the subject's continuous routine factor IX product/dose and at 30 minutes after the factor IX dose is administered (timed from end of infusion). The same continuous routine factor IX product/dose used at the baseline recovery assay should also be used when repeating the assay.
l. 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.
m. Subjects travelling for Visit D may provide their semen sample at Visit L-Final.
n. 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 PT (INR).
o. Serum electrolytes (sodium, potassium), creatinine, creatine kinase, gamma-glutamyltransferase (ɣGT), AST, ALT, alkaline phosphatase (ALP), CRP, albumin, total bilirubin, glucose (non-fasting).
p. Performed for all subjects who have provided separate informed consent for factor IX gene sequencing analysis. Preferably at the screening visit, but otherwise at a later time point during the subject's trial participation.
q. Only if separate informed consent is given by the subject.

**Table 3 Schedule of Events for Efficacy and Safety Evaluation, for Long-Term Follow-up (Visit LTF1 to LTF8)**

| **Trial Period** [a] | **Long-Term Follow-up** [b, c] | | | | | | | | **Additional Visits** [d] |
|---|---|---|---|---|---|---|---|---|---|
| **Visit** | LTF1 | LTF2 | LTF3 | LTF4 | LTF5 | LTF6 | LTF7 | LTF8 | |
| **Month** | 18 | 24 | 30 | 36 | 42 | 48 | 54 | 60 | |
| **Visit window (weeks)** | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | ±2 | |
| Concomitant medication | Continuous | | | | | | | | (x) [d] |
| Collection of bleeding and factor IX use data [e] | x | x | x | x | x | x | x | x | (x) [d] |
| Physical examination | x | x | x | x | | x | | x | (x) [d] |
| Blood and semen sampling (see Table 4) | x | x | x | x | x | x | x | x | (x) [d] |
| Abdominal ultrasound [f] | x | x | x | x | x | x | x | x | (x) [d] |
| 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 | |
| CCI | | x | | x | | x | | x | |
| PROBE [g, h] | | x | | x | | x | | x | |
| Musculoskeletal Ultrasound [i] | | x | | x | | x | | x | |
| Adverse events [j] | Continuous | | | | | | | | |

CCI; h: hour; CCI; CCI; CCI; IMP: investigational medicinal product; CCI; PROBE = Patient Reported Outcomes, Burdes, and Experiences; CCI; CCI

a. This table shows events for the long-term follow-up. Refer to Table 1 for events during screening, lead-in, and treatment + post-treatment follow-up.
b. Each visit in the long-term follow-up is scheduled in relation to the date of IMP dose administration (Visit D), not in relation to the date of its previous visit.
c. For subjects on continuous 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 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 continuous 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 5 half-lives of exogenous Factor IX product use. In case the 5 half-lives washout extends beyond the protocol allowed visit window, the Medpace and uniQure Medical Directors are to be contacted to discuss how to proceed.
d. An additional visit can be performed at any time during the study (long-term follow-up phase) for the purpose of conducting one or more procedures listed in the column "Additional Visits" as per the preference of the Investigator. Hence, "(x)" refers to a procedure that can be performed, if judged relevant by the Investigator.
e. 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. Subjects who are on continuous routine factor IX prophylaxis during the long-term follow-up phase of the trial are required to contact the site staff immediately in case of a bleed and/or factor IX use

different from their routine factor IX prophylaxis, in addition to completing the questions/information requested on the paper diaries to capture all information. The visit frequency is every 26 weeks (6 months) in this long-term follow-up phase, however, there should also be at least quarterly contact (±2 weeks) between the site staff and subject to monitor the proper completion of the study-specific paper diaries, and proper reporting of factor IX usage and bleeding episodes.

f. 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 and up to +1 month after the target visit. Adjustments to the visit timing within this window are to be documented.

g. It is recommended that the CCI [redacted] (CCI [redacted]) 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. The questionnaires are to be completed under site supervision at the site. For those subjects where the LTF2 (Month 24), LTF4 (Month 36), LTF6 (Month 48), and/or LTFU8 (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

h. Applicable only for subjects participating in the optional PROBE questionnaire sub-study. For those subjects where the LTF2 (Month 24), LTF4 (Month 36), LTF6 (Month 48), and/or LTFU8 (Month 60) visits are impacted by COVID-19, the PROBE questionnaire 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.

i. Applicable only for subjects participating in the optional musculoskeletal ultrasound (MSKUS) sub-study. For those subjects where the LTF2 (Month 24), LTF4 (Month 36), LTF6 (Month 48), and/or LTFU8 (Month 60) visits are impacted by COVID-19, scans may be conducted 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 MUSKUS. Adjustments to the visit timing within this window are to be documented.

j. The visit frequency is every 26 weeks (6 months) in this long-term follow-up phase, however there should be at least quarterly contact (±2 weeks) between the site staff and subject to monitor the occurrence of AEs.

**Table 4 Schedule of Events for Laboratory Parameters, for Long-Term Follow-up (Visit LTF1 to LTF8)**

| **Trial Period** [a] | **Long-Term Follow-up** [b, c] | | | | | | | | **Additional Visits** [d] |
|---|---|---|---|---|---|---|---|---|---|
| **Visit** | LTF1 | LTF2 | LTF3 | LTF4 | LTF5 | LTF6 | LTF7 | LTF8 | |
| **Months** | 18 | 24 | 30 | 36 | 42 | 48 | 54 | 60 | |
| **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 | x | (x) [d] |
| Transaminases (AST/ALT) for local monitoring [e] | x | x | x | x | x | x | x | x | (x) [d] |
| **Central Laboratory** | | | | | | | | | |
| One-stage aPTT for factor IX activity | x | x | x | x | x | x | x | x | (x) [d] |
| Chromogenic Assay for factor IX activity | x | x | x | x | x | x | x | x | (x) [d] |
| Factor IX protein concentration | x | x | x | x | x | x | x | x | (x) [d] |
| Anti-factor IX antibodies | | x | | x | | x | | x | (x) [d] |
| Factor IX inhibitors (Nijmegen modified Bethesda assay) [f] | | x | | x | | x | | x | (x) [d] |
| Factor IX Recovery [g] | | | | | | | | | (x) [d] |
| Total (IgM and IgG) antibodies to AAV5 | | x | | x | | x | | x | (x) [d] |
| Neutralizing antibodies to AAV5 | | x | | x | | x | | x | (x) [d] |
| Sampling for vector genome detection | x | x | x | x | x | x | x | x | (x) [d] |
| Blood [h] | x | x | x | x | x | x | x | x | (x) [d] |
| Semen [h] | x | x | x | x | x | x | x | x | (x) [d] |
| Alpha-fetoprotein (AFP) | x | x | x | x | x | x | x | x | (x) [d] |
| Hematology and coagulation parameters [i] | x | x | x | x | x | x | x | x | (x) [d] |
| Serum chemistry parameters [j] | x | x | x | x | x | x | x | x | (x) [d] |

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. This table shows events for the long-term follow-up. Refer to Table 2 for events during screening, lead-in, and treatment + post-treatment follow-up.
b. Each visit in the long-term follow-up is scheduled in relation to the date of IMP dose administration (Visit D), not in relation to the date of its previous visit.
c. For subjects on continuous 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 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 continuous 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 5 half-lives of exogenous Factor IX product use. In case the 5 half-lives washout extends beyond the protocol allowed visit window, the Medpace and uniQure Medical Directors are to be contacted to discuss how to proceed.

d. An additional visit can be performed at any time during the study (long-term follow-up phase) for the purpose of conducting one or more procedures listed in the column "Additional Visits" as per the preference of the Investigator. Hence, "(x)" refers to a procedure that can be performed, if judged relevant by the Investigator.
e. For ALT level increments of at least 2-fold baseline (i.e., Visit D, pre-IMP) and/or > upper limit of normal (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.
f. In case of a positive test for factor IX inhibitors, a re-test should be performed 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.
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 prior to administering the subject's continuous routine factor IX product/dose and at 30 minutes after the factor IX dose is administered (timed from end of infusion). The same continuous routine factor IX product/dose used at the baseline recovery assay should also be used when repeating the assay.
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 (ɣGT), 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 [redacted] (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 14 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

AMT-061 has been developed for the treatment of hemophilia B. AMT-061 is a recombinant adeno-associated viral vector serotype 5 (rAAV5) containing the coding sequence for the Padua variant of the human coagulation factor IX (hFIX-Padua), codon optimized for optimal expression in humans, under control of a liver-specific promoter (also known as AAV5-hFIXco-Padua).

AMT-061 is a derivative of AMT-060, which has been studied in a Phase I/II clinical trial in humans with severe or moderately severe hemophilia B. Both AMT-060 and AMT-061 have the same recombinant AAV5 containing the codon-optimized wild-type human factor IX gene, but the latter incorporates a two-nucleotide change in order to encode the naturally occurring Padua variant of human coagulation factor IX. The FIX-Padua protein differs from the 'wild-type' human factor IX protein by a single amino acid and it is responsible for the observed increased Factor IX activity per unit of dose achieved with AMT-061 as compared to its predecessor AMT-060.

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-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 present in AMT-061, 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 safety profile similar to that observed with AMT-060.

A non-human primate (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 hFIX protein levels, total circulating factor IX activity levels, assessment of vector 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 PT clotting times at the highest dose ($9.0 \times 10^{13}$ genome copies [gc]/kg) of AMT-061 only, without an effect on plasma D-dimer and thrombin-antithrombin levels, suggesting that despite the effect on the clotting times, the overall clotting cascade is functioning within physiological boundaries. The study clearly showed that at a dose of $5 \times 10^{12}$ gc/kg, plasma exposure, liver distribution, liver cell transduction, and transgene expression are similar for both AMT-060 and AMT-061 but the transgene activity is approximately 6 times higher per unit protein for AMT-061 compared to AMT-060. Although the study design only included a direct comparison at $5 \times 10^{12}$ 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 I/II 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 dose confirmation trial (CT-AMT-061-01) with $2 \times 10^{13}$ gc/kg AMT-061. At $2 \times 10^{13}$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 (upper bound of the safety and therapeutic target range is 129%).

The other non-clinical study was a formulation bridging study in wild-type male mice to test AMT-061, manufactured as per process used to manufacture the material for the pivotal clinical trial, in direct comparison with AMT-060, both tested at 3 different concentrations (formulated with or without polysorbate 20). This study showed a similar dose-dependent expression of hFIX protein levels in plasma, supporting equal transgene expression mediated by the two products.

The non-clinical studies with AMT-061 indicated that AMT-061 has a comparable distribution and plasma shedding profile to AMT-060, which was not influenced by the addition of polysorbate 20 to the formulation. The addition of the polysorbate 20 to the formulation had no effect on the safety profile of AMT-060 and AMT-061 in wild-type mice.

Information for AMT-060

A range of *in vivo* studies in wild-type and hemophilia B mice and in 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 hFIX, and to confirm that the hFIX 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 hFIX 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 enzyme 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.

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

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 I/II clinical trial CT-AMT-060-01. The AAV5 capsid and the hFIX gene cassette have been tested individually in other Phase I/II 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, the results of the first in human trial for AMT-060 (CT-AMT-060-01) are summarized in Section 1.3.2.2, and the interim results of the first in human trial for AMT-061 (CT-AMT-061-01) are summarized in Section 1.3.2.3.

#### 1.3.2.1 Preliminary Clinical Evidence for Efficacy and Safety of Components of AMT-061

In Table 5, the most relevant published data and studies conducted with AAV capsids and hFIX 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-061**

| Reference | Capsid | Gene | Promotor | Target tissue | Key Findings |
|---|---|---|---|---|---|
| D'Avola, 2016 | AAV5-based | PBGD | -- | Liver | - No adverse events associated with capsid<br>- Capable of transducing hepatocytes *in vivo* |
| Rangarajan, 2017 | AAV5 | BDD-FVIII | APOE HCR/A1AT | Liver | - Mild asymptomatic transaminase increases in 7 subjects<br>- Capable of delivery that results in clinically relevant transgene expression durable to >1 year |
| Lu et al, 1993 | Retro-viral | hFIX | LTR N2CMV | Autologous skin fibroblasts | - Increase in factor IX activity and improved symptoms in 1 subject<br>- No safety or toxicity concerns |
| Kay, 2000 | AAV2 | hFIX | CMV | Muscle | - Expression of biologically active hFIX<br>- No inhibitors to factor IX |
| Manno, 2003 | AAV2 | hFIX | CMV | Muscle | - Safe incorporation of hFIX into muscle cells<br>- Persistence of vector genome and protein expression to 10 months<br>- 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<br>- No inhibitors to factor IX |

| Reference | Capsid | Gene | Promotor | Target tissue | Key Findings |
|---|---|---|---|---|---|
| Manno, 2006 | AAV2 | hFIX | hAAT-APOE | Liver | - Transient expression of factor IX at clinically relevant doses by transduced hepatocytes<br>- Loss of factor IX expression with ALT increase<br>- No inhibitors to factor IX or T-cell response to the capsid |
| Nathwani, 2011, 2014, 2018 | AAV8 | hFIXco[a] | LP1[a] | Liver | - Successful transduction of hepatocytes resulting in clinically relevant increases in serum factor IX activity<br>- Clinically relevant increases in factor IX activity sustained up to approximately 8 years<br>- Elevated transaminases in 4 subjects; factor IX expression retained in 2 with corticosteroid treatment<br>- No inhibitors to factor IX<br>- No late toxicity |
| George, 2017 | Undisclosed | hFIX-Padua | hAAT-APOE | Liver | - Clinically relevant increases in factor IX activity sustained to >1 year<br>- Elevated transaminases in 2 subjects; partial to full factor IX activity retained with corticosteroid treatment<br>- 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; hFIX: human factor IX; 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-based vector comprising the capsid shell of AMT-060 and containing a functional porphobilinogen deaminase (PBGD) gene tested at varying doses ($5 \times 10^{11}$ to $1.8 \times 10^{13}$ gc/kg; single IV infusion) in eight adult subjects with intermittent acute porphyria. One subject failed screening due to pre-existing AAV5 neutralizing antibodies (NABs). 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 increase (<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 RNA in all six subjects evaluated, suggesting that the AAV5 capsid is able to effectively deliver genetic material to the nuclei of liver cells.

Initial results up to 52 weeks post-treatment have also reported by Rangarajan et al (2017) from a Phase I/II trial of an AAV5-B-domain-deleted factor VIII (FVIII) construct in 9 adults with hemophilia A. In this report, subjects received doses ranging from $6 \times 10^{11}$ to $6 \times 10^{13}$ gc/kg via single IV infusion. No serious adverse events (SAEs) attributable to treatment 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 six experienced mild transient increases in their transaminase levels. One subject experienced a decline in FVIII activity from 227 IU/dL to 52 IU/dL in conjunction with an increase in alanine aminotransferase (ALT) occurring after discontinuation of prophylactic steroids. A second course of corticosteroids was initiated and FVIII subsequently increased. No clear relationship was observed between corticosteroid use and resolution of elevated aminotransferase. All subjects have tapered off corticosteroids and continue to demonstrate increased FVIII activity at 1 year.

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 increases 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 hFIX gene under control of a cytomegalovirus (CMV) promotor/enhancer ($2 \times 10^{11}$ 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 $1.8 \times 10^{12}$ 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 hFIX 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 $2 \times 10^{12}$ gc/kg (Manno et al., 2006). No evidence of increased circulating factor IX activity was obtained at $8 \times 10^{10}$ or $4 \times 10^{11}$ 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 increase 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 incorporated in 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; Nathwani et al., 2018). Subjects received increasing doses ($2 \times 10^{11}$ to $2 \times 10^{12}$ 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 approximately 8 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 8 years post-treatment.

Initial results have been published from a single study showing sustained factor IX expression driven by a gene cassette encoding a Padua variant factor IX (George et al., 2017). Ten subjects received a single IV dose of $5 \times 10^{11}$ gc/kg, resulting in mean steady state factor IX expression of approximately 34% at 52 weeks at time of the report. Two subjects experienced increases 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=31) developed inhibitors to the transgenic hFIX 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. Follow-up of up to 2.5 years from all five subjects in the low dose ($5 \times 10^{12}$ gc/kg) cohort and 2 years follow-up from all five subjects in the high dose ($2 \times 10^{13}$ gc/kg) cohort are currently available (Cohort 1 and 2, respectively; Leebeek et al., 2018; Miesbach et al, 2017). The durability of factor IX protein expression encoded by the codon optimized human coagulation factor IX complementary deoxyribonucleic acid (cDNA) obtained at this point in time is robust and sustainable.

There have been no deaths or AEs that have resulted in discontinuation reported in CT-AMT-060-01. There were a total of 3 SAEs assessed as treatment-related reported, including a febrile episode, elevation of liver enzymes, and elevation of ALT. Fourteen treatment-related AEs were reported in 6 subjects in the first 3.5 months post-treatment. There were no additional treatment-related AEs reported during this additional follow-up period up to 2.5 years post-treatment. Three subjects had mild, temporary ALT elevations 3 to 6 months post-treatment, which resolved with tapering courses of prednisolone with no recurrence. None of the observed ALT increases were associated with any concurrent loss of endogenous factor IX activity or capsid-specific T-cell response. No subject developed inhibitory antibodies to factor IX during the course of the trial.

Following administration of AMT-060, stable factor IX activity and clinically meaningful reductions in bleeds and exogenous factor IX use were observed, with disease severity improved in all subjects. The mean of all endogenous factor IX activity values measured post-treatment over the course of follow-up was 4.9% of normal in Cohort 1 and 7.4% of normal in Cohort 2. 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. Annualized exogenous factor IX use was reduced by ≥82% in Cohort 1 and ≥78% in Cohort 2 compared to pre-treatment. Mean annualized total bleeds decreased by ≥47% in Cohort 1 and ≥62% in Cohort 2.

#### 1.3.2.3 Interim Results for CT-AMT-061-01

Enrollment and dosing in an ongoing dose confirmation Phase IIb trial with AMT-061 (CT-AMT-061-01) has been completed and three subjects have been dosed with $2 \times 10^{13}$ gc/kg of AMT-061. An interim analysis was performed after all subjects had completed 6 weeks of post-treatment follow-up and the interim analysis data set includes a combined 24 weeks of observation. The interim analysis results from CT-AMT-061-01 were reviewed by the Data Monitoring Committee (DMC; see Section 9.12) who recommended that a single dose of $2 \times 10^{13}$ gc/kg of AMT-061 be used in the treatment phase of this Phase III trial.

There have been no deaths, SAEs, or treatment-emergent adverse events (TEAEs) resulting in early discontinuation from CT-AMT-061-01 at the time of the interim analysis. Overall, there have been 13 TEAEs reported in 2 of 3 subjects, including 2 treatment-related TEAEs reported in a single subject (C-reactive protein increased and headache). All TEAEs were mild in severity and the majority (11/13 events) were assessed as not related to study treatment. The system organ class (SOC) with the most reported events was Nervous System Disorders (4 events) and the most common TEAE was headache, reported in 2 subjects, though one subject experienced 6 separate events of pain. One subject had a mild, asymptomatic, and transient increase in liver enzyme levels (specifically, elevated aspartate aminotransferase [AST] levels) which resolved without any additional treatment and was not reported as a TEAE. No T-cell response has been observed in any of the 3 subjects. None of the subjects have developed inhibitory antibodies to factor IX.

All 3 subjects had been diagnosed with severe hemophilia B with corresponding circulating factor IX activity levels <1% of normal. A single dose of $2 \times 10^{13}$ gc/kg of AMT-061 was demonstrated to result in factor IX activity levels of ≥5% as early as 1 week post-treatment with mean ± standard deviation (SD) factor IX activity of 30.6 ± 6.97% (range: 23.9-37.8%) at Week 6 and 28.5 ± 6.42% (range 20.6-37.8%) across visits (factor IX activity was measured using the aPTT assay performed at a central laboratory). Factor IX activity was 37.1% of normal at ten weeks after administration in the first subject, 23.3% of normal at eight weeks following administration in the second subject, and 30.0% of normal at six weeks after administration in the third subject. The second and third subjects had previously screen-failed another gene therapy study due to pre-existing NABs to a different AAV vector. All 3 subjects had evidence of pre-existing NAB activity against adeno-associated viral vector serotype 5 (AAV5).

In the year prior to screening the number of treatment-requiring bleeding episodes ranged from 1 to 5 spontaneous episodes and there was 1 moderate spontaneous bleeding episode in 1 subject in the period between screening and dosing. After dosing, there were no bleeding episodes and the estimated annualized bleeding rate (ABR) was 0. Additionally, none of the subjects have required any infusions of factor IX replacement therapy post-treatment with AMT-061.

## 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. There is also a risk of developing NABs 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.

Three subjects have been treated with $2 \times 10^{13}$ genome copies (gc)/kg AMT-061 in an ongoing dose confirmation Phase IIb trial (CT-AMT-061-01). The initial treatment phase has been completed in this study and an interim analysis based on a minimum of 6 weeks of post-treatment follow-up data for each subject has been performed (see Section 1.3.2.3 for an overview of the interim analysis results). Overall, the dose of $2 \times 10^{13}$ gc/kg AMT-061 was shown to be safe and well tolerated and resulted in mean factor IX activity levels approximately 30% of normal circulating factor IX 6 weeks after dosing.

The results with AMT-061 to date are complemented by robust efficacy and safety data obtained with AMT-060 in the ongoing Phase I/II trial (CT-AMT-060-01) in 10 subjects with hemophilia B who have now been followed for up to 2.5 years after treatment (see Section 1.3.2.2 for an overview of study data). The data demonstrate that subjects achieved clinically relevant and stable factor IX activity levels for up to 2.5 years after treatment with AMT-060. In addition, the AMT-060 treatment remains to be safe and well-tolerated.

The purpose of this Phase III trial is to demonstrate the efficacy of AMT-061 in terms of endogenous factor IX activity and ABR, and to further describe its safety profile. The efficacy and safety results obtained during the Phase IIb study with AMT-061 (CT-AMT-061-01), supplemented with the strong efficacy and safety results obtained during the Phase I/II trial with AMT-060 (CT-AMT-060-01), demonstrate $2 \times 10^{13}$ gc/kg to be the optimal dose for use in this pivotal Phase III trial.

## 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 adeno-associated viral vector (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. In CT-AMT-061-01, one subject had mild TEAEs of headache and paresthesia reported on Day 1, which resolved the same day; the event of headache was assessed as possibly related to study treatment.

As a precaution, subjects in this trial will be monitored for tolerance to the investigational medicinal product (IMP) and detection of potential immediate AEs at the clinical trial site for three 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 NABs. Screening for AAV5 NABs was performed using a bio-assay based on a green fluorescent protein (GFP) reporter gene. In line with previously reported low prevalence for AAV5 NABs (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 NABs 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 NAB 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., $5 \times 10^{11}$ gc/kg versus $5 \times 10^{12}$ gc/kg).

These studies suggested that although AAV5 NABs 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 NABs 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 NAB 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. In the ongoing Phase IIb trial, CT-AMT-061-01, AAV5 NABs were detected in all 3 subjects at Screening. 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

IV 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 5.6.2).

In the CT-AMT-061-01 trial, there were no subjects with an increase in ALT after dosing with AMT-061. A transient increase in AST was noted in one subject with potentially clinically significant levels at 2 × baseline at Week 4 but levels were normal at the next clinic visit and this increase was not reported as a TEAE.

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, feces and semen. In CT-AMT-061-01, vector DNA was detected in all 3 subjects after at least 6, 8, or 10 weeks of follow-up in blood and after 6 weeks of follow-up in semen. In 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 three 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 hFIX 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, 2015; Crudele et al 2015). In CT-AMT-061-01, the formation of factor IX inhibitors was not detected in any of the 3 subjects at 6 weeks after dosing.

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 150 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., 2018). 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 continuous routine use of prophylactic factor IX replacement therapy during the trial.

In an ongoing Phase IIb trial with AMT-061 (CT-AMT-061-01), therapeutic levels of factor IX protein and resultant factor IX activity levels were seen as early as one week. 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. In this trial, subjects will be administered a dose of their usual factor IX product/dose for the factor IX recovery assessment at Visit L-Final and will be permitted to continue their continuous routine factor IX treatment in the first week after dosing.

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 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, Section 6.2.2.7, and Section 6.2.3.4 for new visit windows for CCI questionnaires, musculoskeletal ultrasound (MSKUS), 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, 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

The primary objective is to demonstrate the non-inferiority of AMT-061 ($2 \times 10^{13}$ gc/kg) during the 52 weeks following establishment of stable factor IX expression (months 6 to 18) post-treatment (AMT-061) follow-up compared to standard of care continuous routine factor IX prophylaxis during the lead-in phase, as measured by the ABR.


## 2.3 Safety Objectives

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

# 3 TRIAL DESIGN

## 3.1 Overall Trial Design

This is an open-label, single-dose, multi-center, multi-national trial, with a screening period, a lead-in phase, a treatment plus a post-treatment follow-up phase, and a long-term follow-up phase. Overviews of the trial and its design are presented in Figure 1 and Figure 2.

At the screening visit (Visit S), subjects will be assessed for eligibility and will be instructed in how to record bleeding episodes and use of factor IX replacement therapy in their dedicated electronic diary (e-diary). The approximately 4 week period between screening up to the start of the lead-in phase (Visit L1) is considered a training period where subjects will record their use of factor IX replacement therapy and bleeding episodes in their dedicated e-diary, with the Investigator/study nurse reviewing and evaluating any problems with recording of this data with the subject. The period between screening and the start of the lead-in phase will also be used to wash out subjects from their exogenous factor IX for a pre-defined period of 3 days for regular-acting factor IX products and 10 days for extended half-life factor IX products.

After screening (Visit S), eligible subjects will enter a lead-in phase prior to the start of AMT-061 treatment.

During the lead-in phase, subjects will record their use of factor IX replacement therapy and bleeding episodes in a dedicated e-diary. The length of the lead-in phase is a minimum of 6 months (26 weeks), ending at or before Visit D. Subjects will remain in the lead-in phase until a minimum of 6 months of lead-in data have been collected and it is confirmed that the subject still meets all eligibility criteria. The final visit in this phase (Visit L-Final) will occur approximately 28 days (±7 days) prior to the planned date of IMP dose administration (Visit D). If the ICF is revised during the lead-in phase with important new information that must be shared with the study subjects, the amended ICF will be presented, as required by the Independent Ethics Committees (IECs)/Institutional Review Boards (IRBs), for review and consideration by the subject, and signed re-consent is to be obtained prior to IMP dose administration.

During the lead-in phase, visits are planned as follows:

- Visit L1 will occur after eligibility is confirmed, approximately 4 weeks after screening.
- Alternating monthly clinic visits and follow-up phone calls until a minimum of 6 months of lead-in data have been collected and it is confirmed that the subjects still meet all eligibility criteria (i.e., clinic visits every 2 months; Visit L2 to LX). For follow-up phone calls, an email communication is acceptable as an alternative if the subject cannot be reached, though reasonable efforts for a phone call should be made. The time period between Visit LX and Visit L-Final can be less than 2 months, as long as the total lead-in period is a minimum of 26 weeks.

- The final visit (Visit L-Final) will occur approximately 4 weeks prior to the planned date of IMP dose administration (Visit D).

After the lead-in phase, subjects will receive a single-dose of AMT-061 at Visit D and will be followed for one year (i.e., post-treatment follow-up phase; 52 weeks) to evaluate efficacy and safety. During the post-treatment follow-up, visits are planned as follows:

- Weekly up to Week 12 (Visit F1 to F12)
- Every month from Month 4 to Month 11 (Visit F13 to F20)
- Final visit at Month 12/Week 52 (Visit F-Final)

Following the post-treatment follow-up phase, subjects continue into the long-term follow-up for an additional four years. During the long-term follow-up, visits are planned as follows:

- Every half year (6 months) from 18 to 60 months (4 years; Visit LTF1 to LTF8); however, there should be at least quarterly contact (±2 weeks) between the site staff and subjects to monitor for the occurrence of AEs, proper completion of the study-specific paper diaries, and proper reporting of factor IX usage and bleeding episodes.

Six months after IMP administration, the first secondary endpoint, endogenous factor IX activity at 6 months after AMT-061 dosing, will be analyzed once the last subject has achieved 6 months after AMT-061 treatment. This assessment will be based on clean data and a partially locked database.

Twelve months after IMP administration, the second secondary endpoint, endogenous factor IX activity at 12 months after AMT-061 dosing, will be analyzed and reported via an interim analysis once the last subject has achieved 12 months (52 weeks) after AMT-061 treatment. This assessment will be based on clean data and a partially locked database.

Factor IX expression is anticipated to be stable within 6 months post-dose. After 52 weeks following stable factor IX expression, CCI [redacted]. Data up to that point will be considered locked and will not be changed (with the exception of ending dates and outcomes for continuing events and treatments) without explicit authorization from the Sponsor. Following the 18 months post-dose assessments, subjects will be followed for an additional 3.5 years for evaluation of efficacy parameters and safety. At the end of that 3.5-year period, all safety and efficacy data will be reported in a CSR addendum covering the entire study duration, including the later 3.5-year period.

The overall trial participation will be approximately 5.5 years.

The end of the clinical trial is defined as the point in time the last subject has completed the long-term 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 to accommodate safety concerns and restrictions experienced by individual subjects and sites. In all cases, subjects will be kept informed, via the site staff, as much as possible, of changes to the study and monitoring plans that could impact them.

Discontinuation of subjects from the study post-treatment with AMT-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 supplemental phone 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 e-diary or 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 clinical dose for AMT-060 to proceed to further clinical studies. Although a single administration of AMT-060 at a dose of $2 \times 10^{13}$ gc/kg resulted in successful conversion of clinical phenotype to mild in most subjects (see Section 1.3.2.2), the desired goal is to achieve higher levels of factor IX activity to allow for a more consistent and meaningful clinical response.

AMT-061 is a derivative of AMT-060 that produces the hFIX-Padua variant. Based on NHP studies with AMT-061, as well as data from subjects with the natural Padua mutation, it is expected that, compared to its predecessor, AMT-061 will lead to a higher level of circulating factor IX activity at comparable levels of factor IX protein, thereby increasing the likelihood of alleviating the need for exogenous therapy, including on-demand treatment of traumatic bleeds and selective prevention of a bleed (e.g., because of upcoming physical activity, sports, etc.). At $2 \times 10^{13}$gc/kg, AMT-061 is expected to result in mean Factor IX activity ranges of 40% of normal, ranging between approximately 18-76%. Interim analysis data from the ongoing Phase IIb trial CT-AMT-061-01 demonstrated that AMT-061 was safe and well tolerated and resulted in mean factor IX activity of approximately 30% of normal at 6 weeks after treatment with a dose of $2 \times 10^{13}$ gc/kg (see Section 1.3.2.3).

A dose of $2 \times 10^{13}$ gc/kg for AMT-061 was selected for use in the treatment phase of this study based on interim data from CT-AMT-061-01 and the recommendation from the DMC (see Section 9.12).

The lead-in phase of the trial is used for assessment of the bleedings occuring during standard of care continuous routine factor IX prophylaxis which is part of the primary efficacy parameter. Bleedings and factor IX use data are recorded by the subjects in a dedicated e-diary after a training. The ongoing evaluation of the e-diary data by the Investigator/study nurse and subsequent retraining of the subjects, as necessary, is used to ensure adequate data recording and compliance.

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 efficacy endpoint is as follows:

- ABR comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment)

### 3.3.2 Secondary Endpoints

Secondary efficacy endpoints:

- Endogenous factor IX activity at 6 months after AMT-061 dosing
- Endogenous factor IX activity at 12 months after AMT-061 dosing
- Endogenous factor IX activity at 18 months after AMT-061 dosing
- Annualized consumption of factor IX replacement therapy during the 52 weeks following stable factor IX expression (months 6-18 post-treatment), excluding factor IX replacement for invasive procedures, compared to the lead-in phase
- Annualized infusion rate of factor IX replacement therapy during the 52 weeks following stable factor IX expression (months 6-18 post-treatment), excluding factor IX replacement for invasive procedures, compared to the lead-in phase
- Proportion of subjects remaining free of previous continuous routine prophylaxis during the 52 weeks following stable factor IX expression (months 6-18 post-treatment)
- Comparison of the percentage of subjects with trough factor IX activity <12% of normal between the lead-in phase and after treatment with AMT-061 over the 52 weeks following stable factor IX expression (months 6-18 post-treatment)
- ABR comparison between AMT-061 and prophylaxis for superiority between the lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment)
- Rate of spontaneous bleeding events during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) compared to the lead-in phase
- Rate of joint bleeding events during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) compared to the lead-in phase
- Estimated ABR – during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) – as a function of pre-IMP anti-AAV5 antibody titers using the luciferase based NAB assay (as a “correlation” analysis)
- Correlation of factor IX activity levels during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) with pre-IMP anti-AAV5 antibody titers using the luciferase based NAB assay

- Occurrence of (and resolution of) new target joints during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) and resolution of pre-existing target joints following AMT-061 dosing
- Proportion of subjects with zero bleeds during the 52 weeks following stable factor IX expression (months 6-18 post-treatment)
- CCI during the 12 months following AMT-061 dosing compared with the lead-in phase
- CCI during the 12 months following AMT-061 dosing compared with the lead-in phase

CCI

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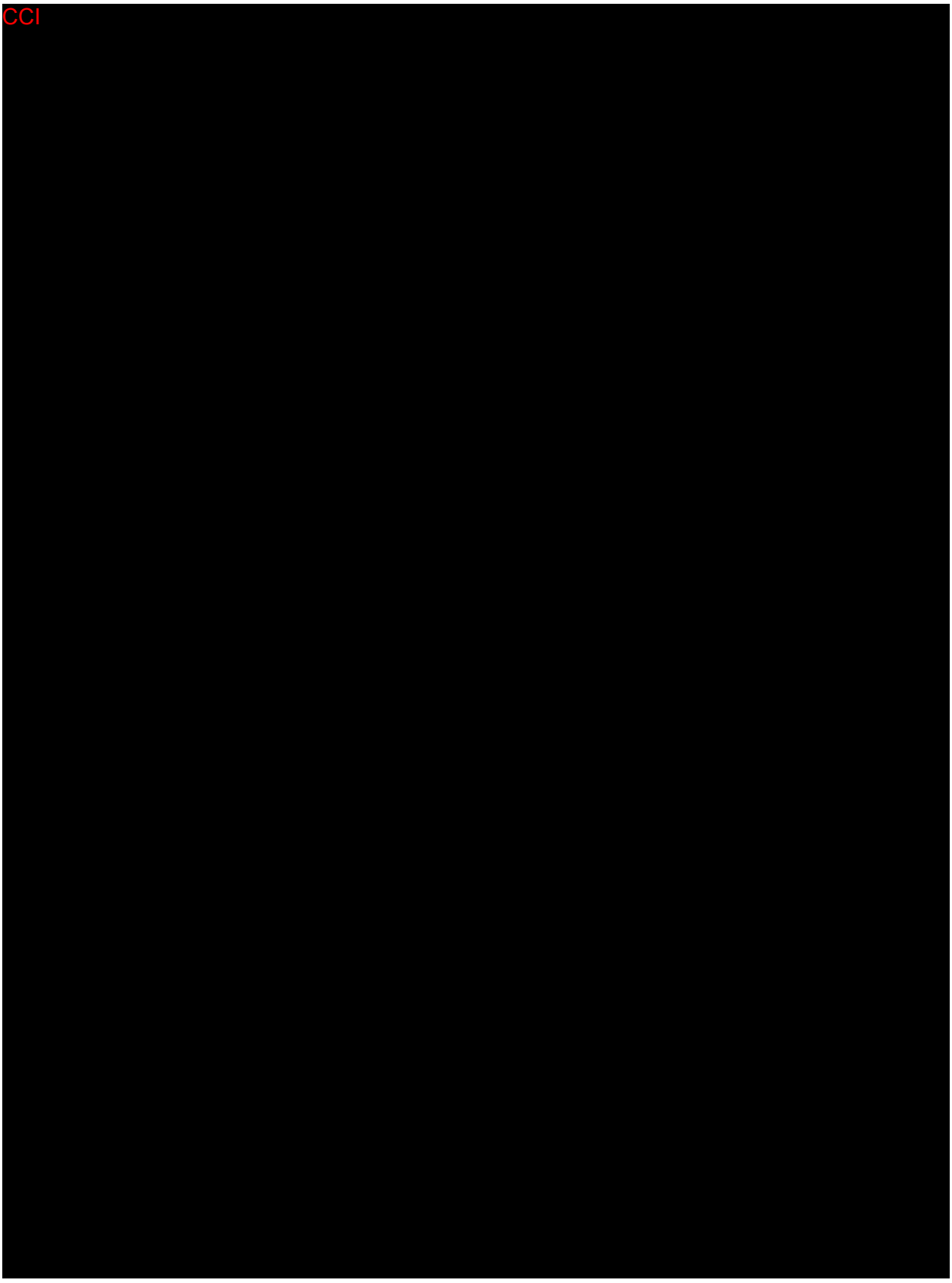

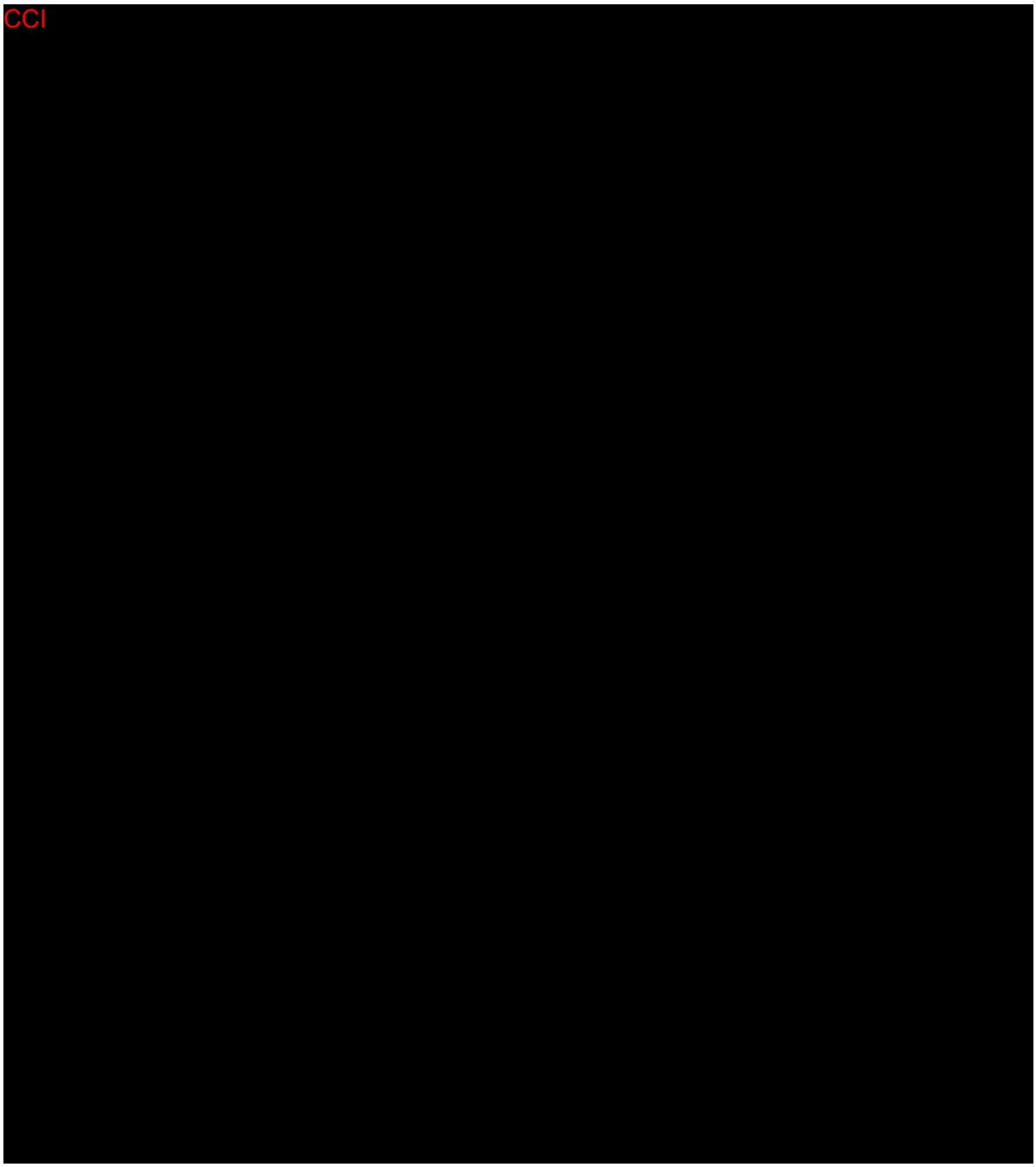


Secondary safety endpoints

- AEs
- Changes in abdominal ultrasound
- Anti-AAV5 antibodies (total [IgM and IgG], NABs)
- AAV5 capsid-specific T cells

- Anti-factor IX antibodies
- Factor IX inhibitors and recovery
- Hematology and serum chemistry parameters
- ALT/AST levels, and corticosteroid use for ALT/AST increases
- Vector DNA in blood and semen
- Inflammatory markers: IL-1β, IL-2, IL-6, IFNγ, MCP-1
- AFP

### 3.3.3 Sub-study Endpoints

- Patient Reported Outcomes, Burdens, and Experiences (PROBE) summary scores and individual item responses.
- MSKUS Sub-study Endpoints
    - Joint Tissue Activity and Damage Exam (J.A.D.E.) scores

## 3.4 Sample Size

The study sample size is constrained by the non-inferiority analysis of the primary endpoint, ABR. Via simulation of ABR under a negative binomial distribution with a yearly rate of 2.4 events for lead-in and 1.9 for post-treatment, with a Pearson correlation of 0.05 for the number of events between the two periods, and with a common negative binomial dispersion parameter of 1.5, a sample size of N=50 will demonstrate non-inferiority with a non-inferiority margin of 1.8 and a power of 82.0%. Therefore, the study should consist of at least 50 analyzable subjects.

Refer to Section 9.11 for further information on sample size justification.

## 3.5 Sites and Regions

It is planned to conduct this trial in approximately 50 sites in the United States of America (USA), European Union (EU), and the United Kingdom (UK).

# 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 are met:

1. Male
2. Age ≥18 years
3. Subjects with congenital hemophilia B with known severe or moderately severe factor IX deficiency (≤2% of normal circulating factor IX) for which the subject is on continuous routine factor IX prophylaxis*
4. >150 previous exposure days of treatment with factor IX protein
5. Have been on stable prophylaxis for at least 2 months prior to screening
6. Have demonstrated capability to independently, accurately and in a timely manner complete the diary during the lead-in phase as judged by the Investigator
7. 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)
8. 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 (including local and central laboratory test results, as specified) are met:

1. History of factor IX inhibitors
2. Positive factor IX inhibitor test at screening and Visit L-Final (based on local laboratory results)
3. Screening and Visit L-Final laboratory values (based on central laboratory results):
   a. ALT >2 times upper normal limit (i.e., upper limit of normal; ULN)
   b. AST >2 times ULN
   c. Total bilirubin >2 times ULN (except if caused by Gilbert disease)
   d. Alkaline phosphatase (ALP) >2 times ULN
   e. Creatinine >2 times ULN

4. Positive human immunodeficiency virus (HIV) serological test at screening and Visit L-Final, not controlled with anti-viral therapy as shown by CD4+ counts ≤200/µL (based on central laboratory results)
5. Hepatitis B or C infection with the following criteria present at screening:
   a. Currently receiving antiviral therapy for this/these infection(s)
      and/or
   b. Positive for any of the following (based on central laboratory results):
      i. Hepatitis B surface antigen (HBsAg), except if in the opinion of the Investigator this is due to a previous Hepatitis B vaccination rather than active Hepatitis B infection
      ii. Hepatitis B virus deoxyribonucleic acid (HBV DNA)
      iii. Hepatitis C virus ribonucleic acid (HCV RNA)
6. Known coagulation disorder other than hemophilia B
7. Thrombocytopenia, defined as a platelet count below $50 \times 10^9$/L, at screening and Visit L-Final (based on central laboratory results)
8. 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
9. Known significant medical condition that may significantly impact the intended transduction of the vector and/or expression and activity of the protein, including but not limited to:
   a. Disseminated intravascular coagulation
   b. Accelerated fibrinolysis
   c. Advanced liver fibrosis (suggestive of or equal to METAVIR Stage 3 disease; e.g., a FibroScan™ score of ≥9 kPa is considered equivalent)
10. Known history of an allergic reaction or anaphylaxis to factor IX products
11. Known history of allergy to corticosteroids
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 ICF in order to maintain the long-term safety follow-up.

Subjects who withdraw from the trial after being dosed with AMT-061 will be requested to complete the same final evaluations (see Section 6.1.4.1) 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 from the trial after enrolling in the Lead-in Phase but who are not dosed will be required to return their e-diary device and will not be requested to complete any final evaluations.

Sufficient subjects will be enrolled to ensure a minimum of 50 subjects in the PP population. It is estimated that at least 56 subjects on continuous routine prophylaxis may be enrolled to ensure there are sufficient subject to achieve this goal.

### 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

### 5.1.1 Investigational Medicinal Product

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 $2 \times 10^{13}$ gc/kg AMT-061.

### 5.1.2 Non-Investigational Medicinal Product

The reference therapy is the prophylaxis factor IX replacement therapy used during the lead-in phase prior to treatment with AMT-061.

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## 5.3 Dosing and Administration

AMT-061 will be administered at a dose of $2 \times 10^{13}$ 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 three hours after dosing.

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 post IMP administration (Visit D), and any new medication/therapy received during the trial.

Concomitant treatment taken as of the date of IMP administration 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 for one year following IMP administration in this trial
- Another gene therapy treatment
- Chronic administration of steroids (oral and/or inhaled)

For any known hepatotoxic medications, other alternatives should be considered. The Investigator is expected to review the concomitant medications on an ongoing basis for these types of medications. Where possible, subjects should be taken off any known hepatotoxic drugs before Visit D. A searchable, comprehensive list of drugs ranked by Drug Induced Liver Injury (DILI) concern (Food and Drug Administration [FDA] Drug Induced Liver Injury Rank [DILIrank] dataset) is available from the following site: https://www.fda.gov/ScienceResearch/BioinformaticsTools/LiverToxicityKnowledgeBase/ucm604985.htm.

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

Subjects are permitted to continue to administer their continuous routine factor IX treatment on the day of dosing (after the pre-IMP assessments are completed) and continue their continuous routine factor IX treatment in the first weeks after dosing to provide sufficient factor IX coverage for the initial days post-treatment. During the post-treatment follow-up visits, endogenous factor IX activity will be assessed. If the endogenous factor IX activity result is ≥5%, continuous routine factor IX prophylaxis will be discontinued and further management will be based on Investigator's clinical judgement and subject preference. Continuation or re-initiation of continuous routine factor IX 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 judgement and subject preference. If endogenous factor IX activity is <2%, continuous routine prophylaxis must be continued or reinstated. Additional on-demand and/or intermittent prophylactic factor IX treatment may be given after treatment with AMT-061, if considered necessary.

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 the blood sample is collected to allow for rapid detection of any elevations in transaminase levels.

For ALT level increments of at least 2-fold baseline (i.e., Visit D, 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 steroid-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 are 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; steroid tapering should be discussed among the Investigator and Medpace and uniQure Medical Directors.

**Table 7 Use of Prednisolone for the Treatment of Transaminase Increase**

| Timeline | Prednisolone dose (mg/day) |
|---|---|
| Week 1 | 60 |
| Week 2 | 40 |
| Week 3 | 30 |
| Week 4 | 30 |
| Maintenance until transaminase level returns to baseline level (Visit D, pre-IMP) | 20 |
| After pre-IMP level has been reached | Reduce daily dose by 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 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 records.

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

Based on entries in the Interactive Web Response System (IWRS) and 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 screening (Visit S), lead-in (Visit L1 to L-Final), and Treatment (Visit D) + post-treatment follow-up (Visit F1 [Week 1] to F-Final [Week 52/Month 12]) refer to the flow charts in Table 1 and Table 2, and for the long-term follow-up (Visit LTF1 [Month 18] to LTF8 [Month 60]) refer to Table 3 and Table 4.

Throughout the study, it will be the aim to draw blood samples at those time points when the subject's factor IX activity level is expected to be at its trough. See Section 6.2.2.3 for further details.

### 6.1.1 Screening (Visit S)

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

At this visit, subjects will be asked to complete the CCI and the CCI will be assessed. It is recommended that each subject should complete the CCI, including PROBE if applicable, after ICF signature, but before other (trial-specific) assessments are performed. Screening assessments will also include medical history, vital signs, physical examination (including height and weight), MSKUS (if applicable), and blood sampling. With prior approval from the Sponsor, screening procedures can occur during the duration of the screening period.

A FibroScan (or suitable alternative) will be performed with liver fibrosis scores collected during the assessment. If possible, steatosis (Controlled Attenuation Parameter [CAP]) scores should also be collected during the assessment. The FibroScan should preferably be done at the screening visit or at an alternative time point, however at Visit L-Final at the latest. If a Fibroscan was performed in the year prior to screening for which fibrosis score is available or if liver biopsy has been performed within the 2 years prior to screening and fibrosis grade was documented, a FibroScan is not necessary.

At this visit, subjects will receive their e-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 the dedicated e-diary. e-diary data will be reviewed on a continuous basis by the Investigator/study nurse. The approximately 4-week period between the screening visit (Visit S) up to the start of the lead-in phase (Visit L1) 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 start of the lead-in phase. 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 who meet an exclusion criterion, or fail to meet an inclusion criterion (screen failures) may only be re-evaluated for participation in the trial after consultation with, and agreement from, the Sponsor.

Separate informed consents are to be taken for blood samples for future research and for factor IX gene sequencing analyses. Factor IX gene sequence analyses will be performed for all subjects that provide consent, even if they already have factor IX gene mutation information available. If the subject provides consent for one or both ICFs, the respective blood samples will be taken during the screening visit.

Separate informed consents are to be taken for the PROBE sub-study, and for the MSKUS sub-study. Assessments will only be performed if the site is participating in the sub-study and the subject has provided consent for the sub-study.

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

From screening onwards, the Investigator or designee will have regular discussions with the subject regarding the importance of a healthy liver before and after receiving a liver directed gene therapy, and factors that might impact liver health (including acetaminophen and alcohol intake). It is recommended that the acetaminophen total daily dose per subject be limited to ≤2 g/day. It is also recommended that subjects on the study should not consume >20 g of alcohol per day. It is recommended that the daily alcohol limit in grams be adhered to most strictly during the first 12 weeks post-IMP. The following stated limit of amount/frequency may help guide the study team to counsel subjects regarding alcohol use in a similar manner across the duration of the trial:

- In the US, one “standard” drink (or a “unit” of alcohol) contains roughly 14 g of pure alcohol, which is found in 12 ounces of regular beer, 5 ounces of wine or 1.5 ounces of distilled spirits (Source: National Institute of Alcohol Abuse and Alcoholism [NIAAA]).

In Europe, alcohol content in a “standard drink” measure differs between countries. On average, they contain between 8 and 13 g of ethanol across Europe. It is recommended that one visits their national consumer information website(s) for further information if necessary (Source: https://www.responsibledrinking.eu/new-page/ ).

While it is recommended that the daily alcohol limit in grams be adhered to most strictly during the first 12 weeks post-IMP, the importance of abstaining from binge-drinking at any point post-IMP administration should be reinforced at every visit.

### 6.1.2 Lead-in Phase (Visit L1 to L-Final)

During the lead-in phase, visits are planned as follows:

- Visit L1 will occur after eligibility is confirmed, approximately 4 weeks after screening.
- Alternating monthly clinic visits and follow-up phone calls until a minimum of 6 months of lead-in data have been collected and it is confirmed that the subjects still meet all eligibility criteria (i.e., clinic visits every 2 months; Visit L2 to LX). For follow-up phone calls, an email communication is acceptable as an alternative if the subject cannot be reached, though reasonable efforts for a phone call should be made. The time period between Visit LX and Visit L-Final can be less than 2 months, as long as the total lead-in period is a minimum of 26 weeks.
- The final visit (Visit L-Final) will occur approximately 4 weeks prior to the planned date of IMP dose administration (Visit D).

The length of the lead-in phase is a minimum of 6 months (26 weeks), ending at or before Visit D. Subjects will remain in the lead-in phase until a minimum of 6 months of lead-in data have been collected and it is confirmed that the subjects still meet all eligibility criteria, with the final visit in this phase (Visit L-Final) occurring approximately 28 days (±7 days) prior to the planned date of IMP dose administration (Visit D).

At Visit L1, blood samples for assessment of factor IX activity and factor IX protein concentration will be taken. Prior to this visit, subjects must have undergone a wash out period from their usual factor IX product/dose. The wash out period will be 3 days for regular-acting factor IX products and 10 days for extended half-life factor IX products.

During the lead-in phase, subjects will record their use of prophylactic factor IX replacement therapy and bleeding episodes in the e-diary. e-diary data will be reviewed on a continuous basis by the Investigator/study nurse, with alternating monthly clinic visits and follow-up phone calls (in line with the trial schedule and with retraining by phone as needed). For follow-up phone calls, an email communication is acceptable as an alternative if the subject cannot be reached, though reasonable efforts for a phone call should be made.

In addition to the subjects' reporting of bleeding episodes in the e-diary, the Investigator or designee will assess each bleeding episode, by describing the reported bleeding episode 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 bleeding, the subject must be called and/or visit the site.

At the clinic visits during this lead-in phase (occurring every 2 months), blood samples will be taken for assessment of factor IX activity, transaminases, and AAV5 antibodies. In addition, the regular discussions with the subject regarding the importance of a healthy liver before and after receiving a liver directed gene therapy, and factors that might impact liver health (including acetaminophen and alcohol intake), will continue.

Subjects will be asked to complete the CCI [redacted] at Visit L3, including PROBE if applicable.

If the ICF is revised during the lead-in phase 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 at Visit L-Final at the latest.

During Visit L-Final, the subject's eligibility will be re-evaluated based on assessments during the lead-in period and at Visit L-Final (for an overview of the inclusion and exclusion criteria 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 as described in Section 6.1.1. The laboratory values obtained at this visit will be used to determine eligibility for dosing and continued participation in the trial. The baseline abdominal ultrasound should be done at Visit L-Final at the latest.

At Visit L-Final, it is recommended that each subject should complete the CCI [redacted], including PROBE if applicable, prior to the interview by the Investigator and/or study nurse and any other trial related procedures. Other assessments at this visit include the MSKUS (if applicable), CCI [redacted], physical examination (including weight), and vital signs evaluation. Additionally, the factor IX recovery assessment is done (see Section 6.2.3.7).

If a subject is travelling to a different site for Visit D, the treatment card should be handed out at the L-Final visit and the subject should be instructed to bring it with him to Visit D. These subjects may also provide their semen sample at Visit L-Final instead of Visit D.

### 6.1.3 IMP Dose Administration (Visit D)

#### 6.1.3.1 Pre-IMP Dose Administration (Baseline, Day 1)

Prior to administration of AMT-061, the Investigator should ensure subject's eligibility (see Section 4.1 and Section 4.2). Laboratory results from Visit L-Final will be used to confirm eligibility for dosing and continued participation in the trial. 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.

At this visit, vital sign measurements will be taken and a physical examination will be performed. Furthermore, baseline blood and semen samples will be collected for efficacy and safety laboratory parameters, serum chemistry, and hematology as detailed in Table 2. Also, for subjects who signed the optional ICF, the sample for future research is taken.

#### 6.1.3.2 IMP Dose Administration

Subjects will receive a single infusion of AMT-061 according to the procedures described in Section 5.3 and in the IMP Handling Manual.

#### 6.1.3.3 Post-IMP Dose Administration (3 hours)

Three hours after completion of the IMP infusion, vital signs are measured and blood samples are taken for C-reactive protein (CRP) measurement and vector genome detection. The subject may leave the clinic after all Visit D, post-IMP assessments have been performed, and the subject has received his subject treatment card. Subjects who travelled to a different site for Visit D should have received their treatment card at the L-Final visit and brought it with them to Visit D.

Subjects are permitted to continue their continuous routine factor IX treatment post-IMP and in the first weeks after dosing to provide sufficient factor IX coverage for the initial days post-treatment.

### 6.1.4 Post-Treatment Follow-up (Visit F1 to F-Final [Week 1 to Week 52/Month 12])

Subjects are followed for a total duration of 52 weeks, with visits weekly for the first 12 weeks, (Visits F1 [Week 1] to F12 [Week 12]), and monthly from Month 4 to Month 12 (Visits F13 [Month 4] to F-Final [Month 12]). With agreement from the Investigator and Sponsor, visits during the post-treatment follow-up period (from Week 1 up until Week 52) that do not include physical examination and/or CCI may be performed at the subject's home by an appropriately qualified and trained nurse. Each home nursing visit is expected to be supplemented by a phone call from the Investigator or designee to the subject to discuss AEs, concomitant medications, and e-diary compliance. Options for how visits may occur to accommodate safety concerns and restrictions due to COVID-19 are described in Section 3.1.1.

During the post-treatment follow-up phase, subjects will continue to record their use of factor IX replacement therapy and bleeding episodes in the e-diary, following the same principles as during the lead-in phase, as described in Section 6.1.2. The Investigator or designee will assess each bleeding episode, by describing the reported bleeding episode 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 bleeding, the subject must be called and/or visit the site. The discussions with the subject on the importance of a healthy liver and factors that might impact liver health (including acetaminophen and alcohol intake), will continue. In addition, use of concomitant medication and occurrence of AEs will be continuously monitored.

Subjects are permitted to continue their continuous routine factor IX treatment in the first weeks after dosing. At Visit F1 (Week 1), 1 week after IMP administration, the subjects will visit the clinic for endogenous factor IX activity assessment (by local and central laboratory). During the post-treatment follow-up visits, factor IX activity levels will be monitored throughout the study. If the endogenous factor IX activity result is ≥5%, continuous routine factor IX prophylaxis will be discontinued and further management will be based on Investigator's clinical judgement and subject preference. Continuation or re-initiation of continuous routine factor IX 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 judgement and subject preference. If endogenous factor IX activity is <2%, continuous routine prophylaxis must be continued or reinstated. Additional on-demand and/or intermittent prophylactic factor IX treatment may be given after treatment with AMT-061, if considered necessary.

Vital signs are measured at each visit in this phase. A physical examination is performed at Visits F1, F2, F4, F6, F12, F13, F15, F17, F19, and F-Final (Weeks 1, 2, 4, 6, and 12, and Month 4, 6, 8, 10, and 12 [Week 52]). Samples for efficacy and safety laboratory parameters and hematology and serum chemistry, are taken at each visit except for the samples for factor IX antibodies and factor IX inhibitors (taken at Visits F6, F12, F15, and F-Final [Weeks 6 and 12, and Months 6 and 12]), semen sampling for shedding (taken at Visits F6, F12, F13, F15, and F-Final [Weeks 6 and 12, and Months 4, 6, and 12]), total (IgM and IgG) antibodies and NABs to AAV5 (taken at Visits F3, F6, F9, F12, F15, and F-Final [Weeks 3, 6, 9, and 12, and Months 6 and 12]), and the blood sample taken for future research (taken at Visits F12 [Week 12] and F-Final [Week 52/Month 12]; see also Table 2). At Visit F15 (Month 6) and Visit F-Final (Month 12/Week 52), it is recommended that each subject should complete the CCI [redacted], including PROBE if applicable, prior to the interview by the trial physician and any other trial related procedures.

For subjects participating in the MSKUS sub-study, assessments will occur at Visit F15 (Month 6) and Visit F-Final (Month 12/Week 52).

Additionally, at Visit F-Final (Month 12/Week 52), an abdominal ultrasound is performed, the CCI is evaluated, the e-diary data devices are collected, the study-specific paper diaries for the long-term follow-up are provided, and the complete efficacy and safety data are analyzed and reported.

#### 6.1.4.1 Early Discontinuation

The procedures listed for the final visit (Visit F-Final [Month 12/Week 52]), with the exception of the distribution of the study-specific paper diaries for the long-term follow-up, must also be performed at early discontinuation (refer also to Section 4.5), but prior to Week 52. In case a subject discontinues after Week 52 but prior to Month 60, he must complete the LTF8 (Month 60) assessments/procedures.

### 6.1.5 Long-Term Follow-up (Visit LTF1 to LTF8 [Month 18 to 60])

During the long-term follow-up, subjects will visit the clinic every 26 weeks (6 months ± 2 weeks). At each visit, the following assessments will be done: review of AEs and concomitant medications, collection of bleeding and factor IX use data, abdominal ultrasound, and sampling for efficacy and safety laboratory parameters. It is recommended that the subject completes the CCI, including PROBE if applicable, at Visits LTF2 (Month 24), LTF4 (Month 36), LTF6 (Month 48), and LTF8 (Month 60), prior to any other trial procedure is performed at the visit. Evaluation of CCI and evaluation of the MSKUS (if applicable) will also be performed at Visit LTF2 (Month 24), LTF4 (Month 36), LTF6 (Month 48), and LTF8 (Month 60). A physical examination will be performed at Visits LTF1 (Month 18), LTF2 (Month 24), LTF3 (Month 30), LTF4 (Month 36), LTF6 (Month 48), and LTF8 (Month 60). There should be an additional contact moment between site staff and subject in between these routine visits to facilitate at least quarterly (± 2 weeks) monitoring of occurrence of AEs, proper completion of the study-specific paper diaries, and proper reporting of factor IX usage and bleeding episodes. 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, 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 a bleed and/or factor IX use in addition to completing the questions/information requested on the paper diaries to capture all information. Subjects who are on continuous routine factor IX prophylaxis during the long-term follow-up phase of the trial are required to contact the site staff immediately in case of a bleed and/or factor IX use different from their routine factor IX prophylaxis, in addition to completing the questions/information requested on the paper diaries to capture all information.

In total, each subject will be followed for approximately 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).

At the end of the long-term follow-up all end of trial procedures, i.e., the LTF8 (Month 60) procedures, as detailed in Table 3 and Table 4, should be performed.

In case of a bleed occurring within 2 weeks of a scheduled LTF visit at which CCI questionnaires should be completed, the study visits should be re-scheduled. The Medpace and uniQure Medical Directors should be contacted to discuss how to proceed in case the visit would need to be pushed out beyond the protocol allowed visit window.

#### 6.1.6 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 assessment 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 6.2.5), or conduct of measurements that were missed at the previous visit.

#### 6.1.7 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 birth year (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. Medical history pertaining to metabolic disorders (diabetes, pre-diabetes, metabolic syndrome), liver disease and hepatitis (metabolic associated fatty liver disease, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis; hepatitis B status, history, and vaccination status; hepatitis C status, history, and eradication details; alcohol use), cancer/tumors (including any family medical history of cancer and specifically hepatocellular carcinoma), will be collected.

Collection of historical medical information regarding hemophilia B is described in Section 6.2.1.4. Information to determine if a subject has liver fibrosis and steatosis will be collected by means of a FibroScan (preferable during screening or at an alternative time point, however at Visit L-Final at the latest). Liver fibrosis scores and steatosis (CAP) scores will be collected during FibroScan assessment. A screening FibroScan assessment is not needed if either 1.) a FibroScan assessment has been performed in the year prior to screening for which CAP scores are available in the source documentation, or 2.) a liver biopsy has been performed within the 2 years prior to the screening for which fibrosis grade is documented and available in the source documentation.

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 medications:

- 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
- Date symptoms were first observed
- Date and value of endogenous factor IX activity assessment demonstrating factor IX activity of ≤2% or sufficient documentation within source documents of severity
- Severity of Hemophilia B
- Family members with a history of factor IX inhibitors
- Arthropathy
- 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 prophylactic factor IX replacement therapy (if applicable)
- Hemophilia B related Surgical History
    - Date of surgery
    - Surgical event

#### 6.2.1.5 History of Bleeding and Factor IX Use

During screening the following historical information regarding bleeding and factor IX use will be collected and recorded in specific modules of the eCRF:

Factor IX use data from one year prior to screening:

- Factor IX product used (registered drug name)
- Type of factor IX replacement therapy (on-demand or prophylaxis)
- Start and stop date
- Dose and frequency of dosing

Bleeding data from one year prior to screening:

- Number of spontaneous bleeding episodes
- Number of traumatic bleeding episodes
- Number of bleeding episodes for which it is unknown if they were spontaneous or traumatic
- Number of joint bleeds

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 International Unites [IU]) used for each procedure

#### 6.2.1.6 Factor IX Gene Sequencing

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). Factor IX gene sequence analyses will be performed for all subjects that provide consent, even if they already have factor IX gene mutation information.

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 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 (Visit S) until Week 52 of the post-treatment follow-up (Visit F-Final), subjects will be asked to record all use of prophylactic and on-demand factor IX replacement therapy in an e-diary. If a subject is not able to enter the details into the e-diary, the site will be able to enter the information in the eCRF as long as the subject provides sufficient source documentation. 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 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 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 (Visit S) until Week 52 of the post-treatment follow-up (Visit F-Final), subjects will record information of bleeding episodes in an e-diary. If a subject is not able to enter the details into the e-diary, the site will be able to enter the information in the eCRF as long as the subject provides sufficient source documentation. 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., pain medication, rest)

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

In case of the occurrence of a 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 schedule of events (Table 2 and Table 4). 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 level is expected to be at its trough.

For subjects on continuous 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 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 continuous routine prophylaxis schedule.

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

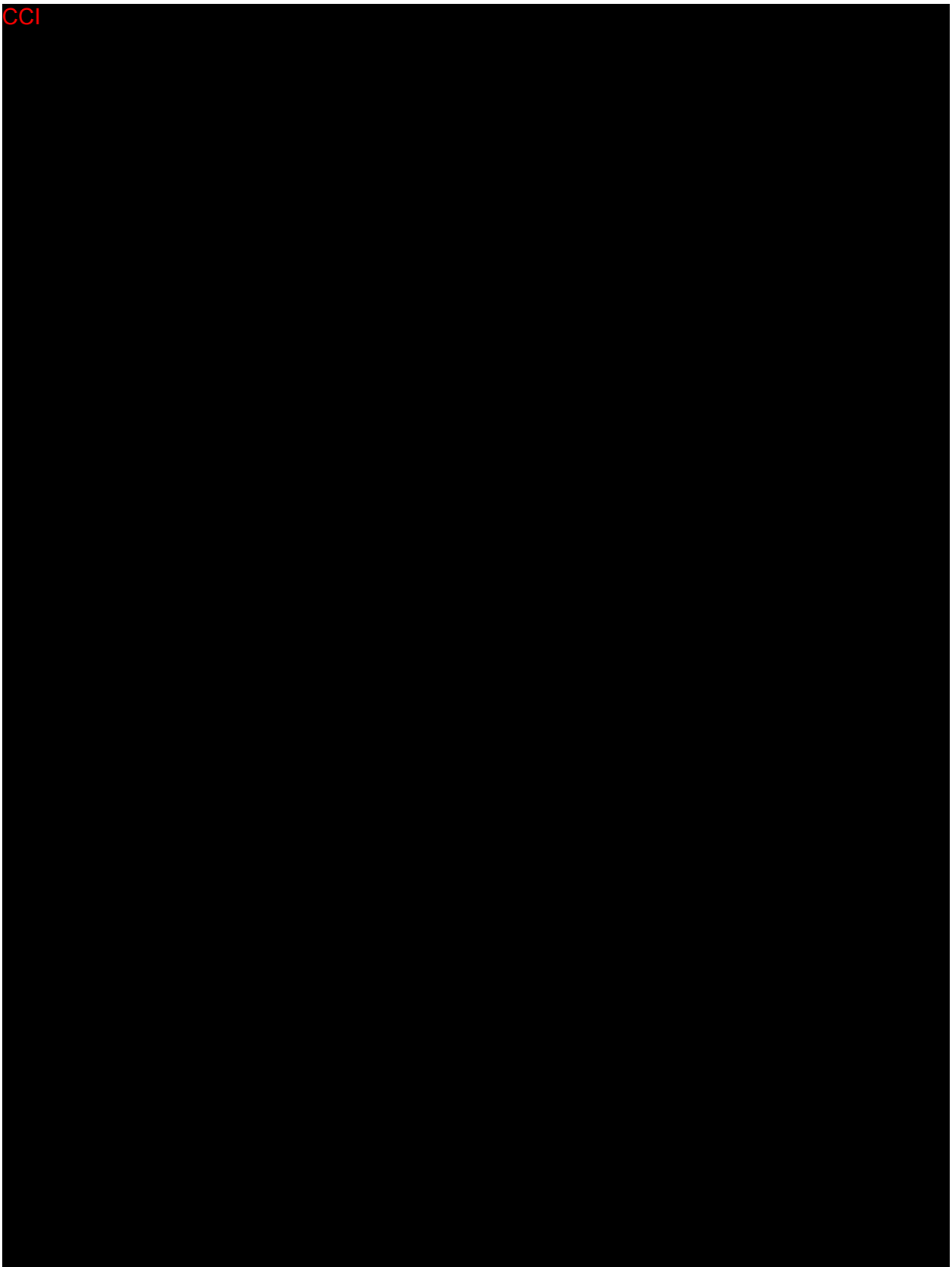
- If a subject uses on-demand factor IX replacement treatment, his upcoming study visit may need to be re-scheduled (to the extent the visit window allows) so that the visit does not take place within 5 half-lives of exogenous Factor IX product use (Table 8). In case the 5 half-lives washout extends beyond the protocol allowed visit window, the Medpace and uniQure Medical Directors should be contacted to discuss how to proceed. Contamination due to infused exogenous factor IX use begins at the date/time of infusion start and ends immediately after the date/time that is 5 times the half-life (of the factor IX medication) after the infusion start date/time. In case the infused factor IX medication is not listed in Table 8, the Medpace and uniQure Medical Directors should be contacted to discuss.

CCI

Factor IX activity will be assessed by using the one-stage aPTT assay (local and central laboratory) and a chromogenic assay (central laboratory).

CCI

CCI



CCI

[redacted]

#### 6.2.2.6 Patient Reported Outcomes, Burdens, and Experiences (PROBE) Questionnaire

The following information applies only to subjects who have volunteered to participate in the optional PROBE Questionnaire Sub-Study.

It is recommended that the PROBE questionnaire will be completed by the subject, after signed informed consent has been obtained and prior to any other visit procedure is initiated (when possible). The Short Form PROBE will be completed at screening (Visit S), Visit L-Final, and Visit LTF8 (Month 60). The PROBE Follow-up Form will be completed at Visit L3, Visit F15 (Month 6), and every year post-baseline (Visits F-Final, LTF2, LTF4, and LTF6 [Months 12, 24, 36, and 48]). The PROBE questionnaire should be completed after the other CCI [redacted]. The PROBE questionnaire is to be completed under the supervision of a qualified and delegated study team member and is not to be given or sent to a subject to complete at home.

For those subjects where Visit F15 (Month 6), Visit F-Final (Month 12), and/or a long-term follow-up visit are impacted by COVID-19, the PROBE questionnaire 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 PROBE questionnaires assessment.

Adjustments to this visit schedule will be documented.

The PROBE Questionnaire is a novel, patient-developed, CCI tool specific to hemophilia and intended to capture clinical outcomes that are considered relevant by patients. The Short Form is the full PROBE questionnaire minus the CCI. The Follow-up Short Form includes select questions from the Short Form (without the CCI).

The objective of this sub-study is to provide data complementary to the compendium of established CCI tools regarding the impact of gene therapy on patient-relevant outcomes and CCI over time.

#### 6.2.2.7 Musculoskeletal Ultrasound (MSKUS)

The following information applies only to subjects who have volunteered to participate in the optional MSKUS Sub-Study.

MSKUS will occur, after signed informed consent has been obtained, at screening (Visit S), Visit L-Final, during the post-treatment follow-up at Visit F15 (Month 6) and Visit F-Final (Month 12), and during the long-term follow-up at Visits LTF2 (Month 24), LTF4 (Month 36), LTF6 (Month 48), and LTF8 (Month 60). If it is not possible to obtain the MSKUS at screening (Visit S), it is allowed to obtain this first MSKUS at a later time point (preferably as soon as possible during one of the lead-in visits). Additional ultrasounds may be collected at the Investigator's discretion. For those subjects where Visit F15 (Month 6), Visit F-Final (Month 12), and/or a long-term follow-up visit are impacted by COVID-19, scans may be conducted 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 MUSKUS.

Adjustments to this visit schedule will be documented.

Repeated bleeds into the joints can result in progressive damage to the joints, culminating in hemophilic arthropathy, mobility restrictions, and possible requirement for surgical repair. This sub-study will provide objective assessment of the effects of receiving gene therapy on the progression of physiological joint damage over time.

Methodology for MSKUS plus scoring of MSKUS will be performed according to the J.A.D.E. protocol. The MSKUSs will be centrally analyzed and results assessed by subject over the course of the study. Full details can be found in the Musculoskeletal Ultrasound Manual.

### 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 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 at screening (Visit S), Visit L-Final, at pre-IMP and post-IMP (3 hours) on Visit D, and at all visits during the post-treatment phase. 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 screening (Visit S), L-Final, Visit D (pre-IMP), during the post-treatment follow-up at Visits F1, F2, F4, F6, F12, F13, F15, F17, F19, and F-Final (Weeks 1, 2, 4, 6, 12 and Months 4, 6, 8, 10, and 12), and during the long-term follow-up at Visits LTF1, LTF2, LTF3, LTF4, LTF6, and LTF8 (Months 18, 24, 30, 36, 48, and 60). Height will only be measured at screening and weight will only be measured at screening and Visit L-Final.

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.

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 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 at Visit L-Final at the latest (to establish baseline status), Visit F-Final, and then every 6 months thereafter as specified in Table 1 and Table 3. For those subjects where Visit F-Final (Month 12), and/or a long-term follow-up visit are 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.

Abnormalities identified on the baseline abdominal ultrasound will be documented in the subject's source documents and on the medical history eCRF. Changes after baseline 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 (or designee) should enter analysis results from the local laboratory, 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 he tested positive for factor IX inhibitors at two consecutive tests, 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 2-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 (timed from end of infusion) of a dose of factor IX, will be performed at Visit L-Final. 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) or at increase in bleeding frequency, as judged by the Investigator.

At each occasion, the subject's continuous routine factor IX product/dose should be administered while at the clinical trial site. A blood sample should be drawn prior to administering the factor IX dose and at 30 minutes after the factor IX dose was administered. The blood sample should preferably be drawn from a vein different from the vein used for factor IX infusion.

The continuous routine factor IX product/dose used for the baseline recovery assay should also be used when the assessment is repeated.

Date of sampling, times of blood sampling (pre- and post-factor IX administration), time of subject's continuous routine factor IX product/dose and identity of subject's continuous routine factor IX product/dose will be recorded.

Factor IX activity for the 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 NABs to AAV5 will be performed at screening (Visit S), Visits L2 through LX, Visit L-Final, Visit D pre-IMP, during the post-treatment follow-up at Visits F3, F6, F9, F12, F15 and F-Final (Weeks 3, 6, 9, 12 and Months 6 and 12), and every year post-baseline (Visits LTF2, LTF4, LTF6, and LTF8 [Months 24, 36, 48, and 60]) 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 bio-assay will be used for NABs 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 baseline (Visit D, pre-IMP) and all visits during the post-treatment phase.

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 (on the visit day or on the day before the visit day). The sampling schedule may be reduced as agreed between the Investigator and subject. As per the inclusion criteria (Section 4.1), the subject must use a condom during sexual intercourse until three consecutive negative samples for AAV5 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 baseline (Visit D) and at specific time points post-baseline, as specified in Table 2 and Table 4 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 9, and as indicated in the schedule of events (Table 2 and Table 4).

**Table 9 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, HBV DNA and HCV RNA |
| Alpha-fetoprotein | Alpha-fetoprotein (AFP) |
| **Local Laboratory** | AST and ALT |

Abbreviations: ALP: alkaline phosphatase; ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; CRP: c-reactive protein; DNA: deoxyribonucleic acid; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HCB: hepatitis C virus; HIV: human immunodeficiency virus; PT: prothrombin time; 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 (or designee) should enter analysis results from the local laboratory, 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 screening will be documented in the subject's source documents and on the medical history eCRF. Changes after 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 [Visit S], baseline [Visit D pre-IMP], Visit F12 [Week 12], and Visit F-Final [Month 12/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 evaluation of subject eligibility (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 those laboratory results needed for treatment decision-making (i.e., laboratory results that inform safety monitoring or management of an individual subject at a site). The Investigator will receive serum chemistry, hematology, coagulation, serology, AFP, inflammatory markers, and gene sequencing results. Laboratory results will be provided at regular intervals for review and sign-off. Note that inflammatory markers will be analyzed by the central laboratory in batches and therefore reporting of these results to the Investigator is delayed. Any abnormality, judged by the Investigator as a clinically relevant worsening since the first measurement, should be reported as an AE, unless the laboratory abnormality is associated with an already reported AE.

Any report of erroneous results from Visit F-Final (Month 12/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).

The Investigator will not be provided with results for one-stage aPTT for factor IX activity, chromogenic assay for factor IX activity, factor IX protein concentration, anti-factor IX antibodies, factor IX inhibitors, factor IX recovery, total (IgM and IgG) antibodies to AAV5, neutralizing antibodies to AAV5, AAV5 capsid-specific T cells, or vector genome detection. Once there have been three consecutive negative results for vector genome detection (in blood and semen), the Investigator will be informed that these samples no longer need to be collected.

### 6.2.6 Volume of Blood to be Drawn From Each Subject

Overall, a total of approximately 2370 mL blood will be drawn from each subject during this trial (excluding additional visits). A maximum of 120 mL will be taken per visit. A maximum amount of 110 mL will be drawn per additional visit.

A minimum of approximately 2000 mL blood will be drawn between screening and the end of the post-treatment follow-up (Visit F-Final [Month 12/Week 52]). During the long-term follow-up, approximately 450 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 number 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 judgement, 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 (i.e., as of ICF signature date) 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, and pregnancies must be reported as described in Table 10 (once the Investigator determines that the event meets the protocol definition for that event).

**Table 10 Timing of Reporting and Follow-up for (Serious) Adverse Events, Adverse Events for Special Notification, and Pregnancies**

| | **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 |

[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, and pregnancy must be reported to CSL Behring.

The 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, and pregnancy 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 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 the 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. Any events of occupational exposure should be reported by the site according to local regulations and procedures.

## 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 judgement 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 required information as per 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; this 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 Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model (SDTM) and serve as the source data from the trial. All 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 (current version at the time of mapping). 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)

All 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 for all study endpoints will be provided in the SAP, which will be finalized prior to database lock. The SAP will include a full description of the availability of data, timing of analyses and reporting, and will include templates for the tables, figures, and listings to be provided.

Any agreed deviations from the SAP will be justified in the CSR.

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

## 9.2 Interim Analyses

The first secondary endpoint, endogenous factor IX activity at 6 months after AMT-061 dosing, will be assessed once the last subject has achieved 6 months after AMT-061 treatment. This assessment will be based on clean data and a partially locked database.

The second secondary endpoint, endogenous factor IX activity at 12 months after AMT-061 dosing, will be assessed once the last subject has achieved 12 months after AMT-061 treatment. This assessment will be based on clean data and a partially locked database.

## 9.3 Final Analysis

Factor IX expression is anticipated to be stable within 6 months post-dose. After 52 weeks following stable factor IX expression, CCI [redacted]. Data up to that point will be considered locked and will not be changed (with the exception of ending dates and outcomes for continuing events and treatments) without explicit authorization from the Sponsor.

Following the 18 months post-dose assessments, subjects will be followed for another 3.5 years for evaluation of efficacy parameters and safety. At the end of that 3.5-year period, all safety and efficacy data will be reported in a CSR addendum covering the entire study duration, including the later 3.5-year period.

The CSR and CSR addendum 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

The FAS (Full Analysis Set) will include all subjects who are enrolled, entered the lead-in phase, are dosed with AMT-061, and provide at least one efficacy endpoint assessment subsequent to AMT-061 dosing. The FAS population will be the primary population for all efficacy statistical analyses.

The PP population will include all subjects from the FAS population who adhere to a stable and adequate prophylaxis use during the lead-in phase, who complete at least 18 months of efficacy assessments (52 weeks after achieving stable factor IX expression) for the 18-month (data cut) analysis, who complete at least a full year of efficacy assessments for the 12 month (data cut) analysis, or who complete at least 6 months of efficacy assessments for the 6 month (data cut) analysis, and who have no major protocol deviations that impact the interpretation of efficacy. The PP population will be used for sensitivity analyses. Protocol deviations that impact the interpretation of efficacy include unwillingness to stop continuous routine factor IX prophylactic treatment after receipt of AMT-061. Detailed rules for exclusion of data from the PP analysis will be established.

The lead-in safety population will consist of all subjects enrolled into the lead-in period. The post-treatment safety population will consist of all subjects who receive AMT-061, irrespective of any protocol deviations. Period-specific safety tabulations will use the period-specific safety population for the “N” and denominator (for percentages). The safety population will consist of all subjects who are in either the lead-in safety population or the post-treatment safety population.

The screen failure population will include all subjects who were screened but never entered the lead-in period.

The lead-in discontinuers population will include all subjects who entered the lead-in period but discontinued from the study prior to AMT-061 dosing.

## 9.5 Subject Disposition

A disposition table for CT-AMT-061-02 for all subjects will be provided. This tabulation will include the number of subjects who were not treated, who received the study treatment, who discontinued from the study, and who completed the study. The number and percentage of subjects included in the FAS, PP, and Safety populations will also be tabulated. The reason for exclusion from the FAS, PP, and Safety populations will be summarized. Reasons for premature discontinuation from study treatment (i.e., being treated with only a partial dose of AMT-061) will be summarized for the Safety population.

The data on subject disposition, protocol deviations (including those related to COVID-19), and informed consent will be listed.

## 9.6 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics will be presented for the FAS, PP, and Safety populations. For quantitative variables, all summaries will include the number of non-missing observations, mean, median, SD, minimum, and maximum. For the qualitative variables, the summaries will include the number and percentage of subjects in each category or level. All data will be included in listings.

Medical history will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA), summarized using the number of observations and percentages of subjects reporting each category, and listed for each subject.

A full description of demographic variables will be included in the SAP.

## 9.7 Investigational Product Exposure

Listings for exposure to IMP will be provided showing the date of exposure and dose received.

The time of subject's routine factor IX product/dose, incremental recovery, maximum concentration, and identity of subject's routine factor IX product/dose will be listed.

## 9.8 Prior and Concomitant Medication

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

## 9.9 Efficacy Analyses

All efficacy analyses will be performed for the FAS and PP populations. Statistical analysis will be performed and 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.

The primary efficacy analysis will be completed using the FAS population. The analysis using the PP population is considered to be a sensitivity analysis.

### 9.9.1 Primary Efficacy Endpoints

The primary efficacy endpoint is as follows:

- ABR comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment)

*Primary efficacy analysis for ABR*

ABR will be determined for the lead-in period and post-treatment period (for the 52 weeks following stable factor IX expression [months 6-18 post-treatment]). Analysis of the number of reported bleeding events will be performed using a repeated measures (RM) generalized estimating equations (GEE) negative binomial regression model accounting for the paired design of the trial with an offset parameter to account for the differential collection periods. An unstructured covariance matrix will be employed. If the model fails to converge, then a compound symmetry covariance structure will be used. The model will include the treatment (i.e., period) as a categorical variable. If convergence is not attained, then initial parameter estimates will be provided. The estimated rate ratio, one-sided 97.5% Wald confidence interval, and the corresponding p-value will be determined. The upper limit of the resultant confidence interval of the rate ratio will be compared to the non-inferiority margin of 1.8. If the upper limit is less than 1.8, then non-inferiority will be declared.

For the data cut at 52 weeks following stable factor IX expression (18 months post-treatment), ABR counts over the 52 weeks following stable factor IX expression (months 6-18 post-treatment) will be used in the analysis. For data cuts prior to the post-treatment 18-month data cut, to allow time for AMT-061 to become active and to allow the subject the opportunity to stop the lead-in prophylactic Factor IX therapy, ABR counts beginning at Day 21 (of the post-treatment period) will be used in the analysis.

The post-AMT-administration time at risk of (having) a bleeding event is the subject's time on the study between stable factor IX expression (Month 6; between Day 21 for pre-Month 18 data cuts) and the time that is 52 weeks following stable factor IX expression (18 months; that is one year for the 12-month data cut), the time of study completion, or the time of early withdrawal from the study, whichever is earlier. Any bleeds prior to stable factor IX expression (Month 6; to Day 21 for pre-Month 18 data cuts) of the post-treatment period are not considered in the analysis. Events from the entire lead-in period will be counted, and the entire lead-in period is considered to be time at risk.

In the analysis, any person-time during the post-treatment period within 5 half-lives subsequent to exogenous factor IX use will not be counted in the time at risk of (having) a bleeding event. Any bleeds occurring on or after stable factor IX expression (post-treatment Month 6; on or after Day 21 for pre-Month 18 data cuts) should still be counted as events, even if they occurred during a time interval of "contamination".

The main population for analysis will be the FAS.

### 9.9.2 Secondary Efficacy Endpoints and Order of Testing of Primary and Secondary Endpoints

Secondary endpoints of the trial will focus on investigating the effect of $2 \times 10^{13}$ gc/kg AMT-061 on endogenous factor IX activity, assessment of annualized consumption (and infusion rate) of factor IX replacement therapy, remaining free of previous continuous routine prophylaxis, assessment of trough factor IX activity, bleeding events, estimated ABR as a function of pre-IMP anti-AAV5 antibody titers using the luciferase based NAB assay (as a "correlation" analysis), correlation of factor IX activity levels and observed anti-AAV5 antibody titers using the luciferase based NAB assay after AMT-061 dosing, occurrence and resolution of target joints, CCI , and CCI .

Secondary efficacy endpoints are presented in Section 3.3.2.

*Efficacy analyses for factor IX activity*

For the factor IX activity analyses, the change in uncontaminated endogenous factor IX activity levels (by the one-stage aPTT assay) at 6 months, 12 months, and 18 months following a single treatment with AMT-061 will be assessed once the last subject has achieved 6 months, 12 months, and 18 months after AMT-061 treatment, respectively. The methodology, as described below for assessment after 18 months, will also be used for the 6 month and 12 month analyses.

The change from baseline in factor IX activity (factor $IX_{DIFF}$) will be tested:

- $H_o$: *factor* $IX_{DIFF} = 0$ *(no effect of treatment)*
- $H_1$: *factor* $IX_{DIFF} > 0$

The hypothesis that factor $IX_{DIFF} = 0$ (i.e., that the change from baseline is zero) will be tested and a one-sided p-value $\leq 0.025$ will be regarded as statistically significant.

The change from baseline in factor IX activity (percent of normal) at Month 18 will be analyzed using a RM linear mixed model. The baseline factor IX activity will be imputed as described in the SAP. If a subject has zero uncontaminated central-laboratory post-AMT-061 factor IX activity values, the factor IX activity at Month 18 (and at any other post-AMT planned assessment time point that is to be used in the analysis) will be imputed based on the subject's historical hemophilia B severity as documented on the CRF in a manner identical to that used for baseline factor IX activity. The model will include visit as a categorical covariate. A Toeplitz covariance matrix will be used to model correlation within a subject. If the Toeplitz model fails to converge, then a first-order autoregressive (AR(1)) structure will be used instead. In the AR(1) model, subject will be included as a random effect. If the AR(1) model also fails to converge, then subject will be modeled as a random effect in the absence of an AR(1) model. If convergence is still not attained, then initial parameter estimates will be provided. A contrast will be used to carry out the comparison at Month 18. The change from imputed baseline at

Month 18, the two-sided 95% confidence interval for the mean change, and the corresponding p-value for the comparison to zero will be obtained from the model and provided in a table.

To allow time for AMT-061 to become active and to allow the subject the opportunity to stop the lead-in prophylactic factor IX therapy, factor IX levels beginning with the Week 3 assessment will be used in the analysis. Visits post-AMT-061 that are within 5 half-lives of exogenous factor IX use are considered contaminated and will also be excluded from this analysis. For the 6-month analyses, a less refined contamination rule was used, whereby the date of exogenous factor IX infusion and the subsequent 9 days (10 discrete calendar days in total) were considered to be days of contamination with factor IX. The 12 month and 18 month analyses will use the more refined definition of contamination, based on 5 half-lives.

The main efficacy analysis for factor IX activity will be completed using the FAS population. The analysis using the PP population is considered to be a sensitivity analysis. Sensitivity analyses will be conducted to account for missing data and will be specified in the SAP.

*Testing of Efficacy Endpoints*

Formal statistical testing of the efficacy endpoints will be performed using the closed testing principle (for Type I error control for multiple testing). Due to the closed testing principle, no correction for multiplicity is necessary. All endpoints will be tested for superiority at a one-sided alpha level of 0.025 (except as otherwise noted). Superiority and non-inferiority testing will be accomplished using the FAS population. Fixed sequential testing will be performed using a hierarchical approach and will be continued until a non-significant result is obtained (except as otherwise noted).

The primary and secondary endpoints will be analyzed in the following order:

1. ABR comparison between AMT-061 and prophylaxis for non-inferiority between lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment; primary efficacy endpoint)
2. Endogenous factor IX activity at 6 months after AMT-061 (first secondary efficacy endpoint)
3. Endogenous factor IX activity at 12 months after AMT-061 (second secondary efficacy endpoint)
4. Endogenous factor IX activity at 18 months after AMT-061 (third secondary efficacy endpoint)
5. Annualized consumption of factor IX replacement therapy during the 52 weeks following stable factor IX expression (months 6-18 post-treatment), excluding replacement for invasive procedures, compared to the lead-in phase (secondary efficacy endpoint)
6. Annualized infusion rate of factor IX replacement therapy during the 52 weeks following stable factor IX expression (months 6-18 post-treatment), excluding replacement for invasive procedures, compared to the lead-in phase (secondary efficacy endpoint)

7. Comparison of the percentage of subjects with trough factor IX activity <12% of normal between the lead-in phase and after treatment with AMT-061 over the 52 weeks following stable factor IX expression (months 6-18 post-treatment; secondary efficacy endpoint)
8. ABR comparison between AMT-061 and prophylaxis for superiority between the lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment; secondary efficacy endpoint)
9. Rate of spontaneous bleeding events during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) compared to the lead-in phase (secondary efficacy endpoint)
10. Rate of joint bleeding events during the 52 weeks following stable factor IX expression (months 6-18 post-treatment) compared to the lead-in phase (secondary efficacy endpoint)
11. CCI [redacted] during the 12 months following AMT-061 dosing compared with the lead-in phase (secondary efficacy endpoint)
12. CCI [redacted] during the 12 months following AMT-061 dosing compared with the lead-in phase (secondary efficacy endpoint)

Simultaneous one-sided 97.5% confidence intervals based on a graphical approach to multiple testing will be provided for the Type I error controlled efficacy endpoints as a supportive analysis. For endpoints for which an increase is favorable, the lower one-sided 97.5% confidence bound will be provided; for endpoints for which an increase is unfavorable, the upper one-sided 97.5% confidence bound will be provided. For any data cuts (i.e., analysis times) that are not the main data cut for a given endpoint, the p-values and CIs will be considered to be descriptive rather than inferential.

All data will be listed.

A full description of the secondary analyses will be included in the SAP.

CCI [redacted]

### 9.9.4 Optional Sub-study Endpoints

Optional sub-study endpoints are presented in Section 3.3.3.

The analysis for the optional PROBE Questionnaire sub-study will be described in the SAP. There will be a separate SAP document for the statistical analysis of the optional MSKUS sub-study results.

Analysis will be based on the FAS population for the set of subjects participating in the respective sub-study. Any subject with at least one assessment of the sub-study endpoint will be considered to be participating in the respective sub-study.

## 9.10 Safety Analyses

Secondary safety endpoints to be analyzed are presented in Section 3.3.2.

All TEAEs 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) 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, definitely related/probably related/possibly related/unrelated TEAE, Serious TEAE, TEAE for special notification, and 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.

Other safety data will be presented using graphical displays, as applicable, descriptive statistics (including change from baseline, if applicable), and/or individual data listing.

The number of days until vector DNA can no longer be detected in semen and blood will be tabulated. The number of days is calculated using the date of collection of the first of three consecutive negative sample for each matrix.

All safety analyses will be based on the Safety population.

## 9.11 Sample Size Justification

The study sample size is constrained by the non-inferiority analysis of the primary endpoint, ABR.

Based on a literature search of trials in a similar clinical setting and the same underlying disease, as well as the previous AMT-060 Phase I/II trial, a non-inferiority margin of 1.8 is assessed for the rate ratio of ABR between AMT-061 (post-treatment) and factor IX prophylaxis (lead-in). For establishing the non-inferiority margin, an ABR of 2.4 between factor IX prophylaxis and placebo treatment has been assumed. Via simulation of ABR under a negative binomial distribution with a yearly rate of 2.4 events for lead-in and 1.9 for post-treatment, with a Pearson correlation of 0.05 for the number of events between the two periods, and with a common negative binomial dispersion parameter of 1.5, a sample size of N=50 will demonstrate non-inferiority with a non-inferiority margin of 1.8 and a power of 82.0%. Therefore, the study should consist of at least 50 analyzable subjects.

Given the sample size needed for ABR, this will produce a power >95% for the secondary statistical analysis of endogenous factor IX activity.

## 9.12 Data Monitoring Committee

A 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, CT-AMT-061-02, is to monitor the safety of the subjects throughout the trial.

The DMC will also be involved in monitoring the safety of the subjects in the dose confirmation trial, CT-AMT-061-01, as well as to evaluate response to the treatment in terms of factor IX activity levels. The DMC has assessed whether observed factor IX activity levels in the subjects treated with AMT-061 in the dose confirmation trial are within an expected range (as defined in the DMC charter) to determine if the administered dose in the dose confirmation trial is suitable for administration in this trial. If the DMC had determined that the observed response was not within the expected range, or they did not observe enough consistency of effect, to proceed to dosing in this trial, they could have elected to recommend up to three more subjects be treated at the same dose or recommend a second dose be studied in the dose confirmation trial. The DMC reviewed the 6-weeks post-treatment interim analysis results from the dose confirmation trial and confirmed that the dose of $2 \times 10^{13}$ gc/kg for AMT-061 met the intended safety and efficacy profile and that the same dose should be used in the treatment phase of this trial.

In addition, the DMC will assess whether there is an impact of pre-existing NAB titers on clinical outcome following AMT-061 (per criteria defined in the DMC charter). Should the DMC determine that there is a recognizable impact of a certain titer and above, they can recommend institution of an exclusion criterion based on these titers for further enrollment in the study.

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 CRFs 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; this data 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 [redacted]

#### 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 [redacted], CCI [redacted] 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 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), the PROBE Questionnaire Sub-study (Section 6.2.2.6), and the MSKUS Sub-study (Section 6.2.2.7).

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).

IMP 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

Treatment cards will be handed out at Visit D. If a subject is traveling to a different site for Visit D, this card should be handed out at the L-Final visit and the subject should be instructed to bring it with him to Visit D.

## 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, 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 data, and possibly 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 have adequate resources and facilities to conduct the study

## 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.
- Storage, secondary packaging, shipping, and EU Qualified Person (QP) release of IMP
- 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<br>PPD | Central Laboratory:<br>Coagulation<br>Chemistry<br>Inflammatory markers/cytokines<br>Serology<br>Hematology<br>CD4 counts<br>PBMC isolation for AAV5-capsid specific T-cell response (up until June 2019) |
| PPD<br>PPD | Referral testing:<br>Vector genome detection (blood and semen) |
| PPD<br>PPD | Referral Testing:<br>Factor IX Activity: one-stage aPTT, chromogenic assay<br>Factor IX protein concentration<br>Factor IX inhibitors<br>Anti-factor IX antibodies<br>IgG antibodies AAV5/IgM antibodies AAV5 |
| PPD<br>PPD | Referral Testing:<br>PBMC isolation for AAV5-capsid specific T-cell response (starting July 2019)<br>AAV5-capsid specific T-cell<br>NABs to AAV5 |
| PPD<br>PPD | Referral Testing:<br>Factor IX genetic sequencing |
| PPD<br>PPD | Confirmation Testing:<br>HIV-1/2 Antibody differentiation test |

## 13.2 Summary of Changes in Current Amendment

| **Protocol Amendments** | | |
|---|---|---|
| **Summary of Changes Since Last Version of Approved Protocol** | | |
| **Amendment Number:**<br>7.0 (Version 8.0) | **Amendment Date:**<br>07 Feb 2022 | **Global/Country/Site-Specific:**<br>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<br>Section 7.5<br>Section 7.6 |
| The identity and details for the uniQure Medical Director were updated. | | Emergency Contact Information |
| It was clarified that for subjects only using on-demand Factor IX replacement therapy, study visits may need to be re-scheduled so that the visit does not take place within 5 half-lives of exogenous Factor IX product use. Table 8 “Half-Life for Infused Exogenous Factor IX Medical by Name” was also added to clarify half-lives for infused exogenous factor IX medications. | | Table 1<br>Table 2<br>Table 3<br>Table 4<br>Section 6.2.2.3<br>Table 8 |
| Footnote notation was adjusted for clarity in Table 4 “Schedule of Events for Laboratory Parameters, for Long-Term Follow-up (Visit LTF1 to LTF8)” for sampling for vector genome detection blood and semen. | | Table 4 |
| It was clarified that in case of a bleed occurring within 2 weeks of a scheduled LTF visit at which CCI should be completed, the study visits should be rescheduled. | | Section 6.1.5<br>Section 6.2.2.4 |
| 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<br>Section 10.3.1 |

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| Due to the addition of Table 8, the previous Table 8 is now Table 9. Due to the addition of Table 8, the previous Table 9 is now Table 10. | Table 9<br>Table 10 |
| The phrase “using the Common Terminology Criteria for Adverse Events severity grades” was removed from Section 9.10 | Section 9.10 |
| 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

| **Protocol Amendments** | | |
|---|---|---|
| **Summary of Changes** | | |
| **Amendment Number:**<br>6.0 (Version 7.0) | **Amendment Date:**<br>28 Jun 2021 | **Global/Country/Site-Specific:**<br>Global |
| **Description of Change** | | **Section(s) Affected by Change** |
| Study objectives and endpoints were updated, based on input from the FDA.<br>The primary objective and endpoint was updated to focus on ABR, 52 weeks following establishment of stable factor IX expression (months 6 to 18 post-treatment).<br>The previously primary objectives and endpoints related to endogenous factor IX activity at 6 months and 12 months were moved to be the first and second secondary efficacy endpoints. Endogenous factor IX activity at 18 months after AMT-061 dosing was added as the third secondary efficacy endpoint. Estimated ABR as a function of pre-IMP anti-AAV5 antibody titers using the luciferase based NAB assay was added as a secondary efficacy endpoint (as a "correlation" analysis). CCI [redacted] | | Synopsis<br>Section 2<br>Section 3.3<br>Section 9.9<br>Section 9.10 |
| It was clarified that the FAS population would be used for all efficacy statistical analyses, with the PP population used for sensitivity analyses. The definition of the PP population was updated to clarify the timepoints used to identify the population for various data cuts. | | Synopsis<br>Section 9.4 |
| To align with the updated endpoints, the statistical analyses were also reordered and timing clarified, including the order of endpoints for the fixed sequential testing of the primary and secondary efficacy endpoints. | | Synopsis<br>Section 9.9.1<br>Section 9.9.2 |

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| As the primary objective was adjusted to focus on ABR, the description of the number of subjects, the sample size, and sample size justification, was similarly updated; the sample size remained at 50 subjects. | Synopsis<br>Section 3.4<br>Section 9.11 |
| Details on the interm and final analyses were updated. The timing of the CSR was clarified to occur after 18 months post-treatment with AMT-061, instead of after 12 months. An interim analysis would still occur after 12 months to assess endogenous factor IX activity. The study period included in the CSR addendum was also clarified. | Synopsis<br>Section 3<br>Section 9.2<br>Section 9.3 |
| Clarification that during the long-term follow up, quarterly contact (±2 weeks) should occur to monitor for AEs, proper completion of study-specific patper diaries, and proper reporting of factor IX usage and bleeding episodes | Synopsis<br>Table 3<br>Section 3<br>Section 6.1.5 |
| It was clarified that the calculation for the number of days until vector DNA can no longer be detected in blood and semen would be based on the first of three consecutive negative samples. | Synopsis |
| Additional detail on medical history to be specifically collected was added. | Section 6.2.1.2 |

| Summary of Changes | | |
|---|---|---|
| **Amendment Number:** 5.0 (Version 6.0) | **Amendment Date:** 04 Feb 2021 | **Global/Country/Site-Specific:** Global |
| **Description of Change** | | **Section(s) Affected by Change** |
| Update to uniQure Clinical Development and Pharmacovigilance signee. | | Clinical Trial Protocol Signature Page |
| Update to uniQure Medical Director details. | | Emergency Contact Information |
| The frequency of abdominal ultrasounds in the long-term follow-up phase was increased from yearly to every 6 months. The abdominal ultrasound was added as a procedure that could be performed at an unscheduled visit during the long-term follow-up, if judged relevant by the Investigator. The permitted visit window for abdominal ultrasounds conducted at Visit F-Final and in the long-term follow-up phase when the visit is impacted by COVID-19 was adjusted to be up to -1 month prior to the target visit and up to +1 month (previously up to +2 months) after the target visit. | | Synopsis<br>Table 1<br>Table 3<br>Section 6.1.5<br>Section 6.2.3.4 |
| Clarification was added that subjects who are on continuous routine factor IX prophylaxis during the long-term follow-up phase of the trial are required to contact the site staff immediately in case of a bleed and/or factor IX use different from their routine factor IX prophylaxis, in addition to completing the questions/information requested on the paper diaries to capture all information. | | Synopsis<br>Table 3<br>Section 6.1.5 |
| For CCI [redacted], the option to be considered a missed assessment if the questionnaires cannot be completed within the allowed visit window when a visit is impacted by COVID-19, was removed. It was clarified that adjustments to the visit timing within the allowed visit window are to be documented. | | Table 1<br>Table 3<br>Section 6.2.2.4 |
| For the PROBE questionnaire sub-study, the option to be considered a missed assessment if the PROBE questionnaire cannot be completed within the allowed visit window when a visit is impacted by COVID-19, was removed. It was clarified that adjustments to the visit timing within the allowed visit window are to be documented. | | Table 1<br>Table 3<br>Section 6.2.2.6 |

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| For the MSKUS sub-study, the option to be considered a missed assessment if the scans cannot be completed within the allowed visit window when a visit is impacted by COVID-1, was removed. It was clarified that adjustments to the visit timing within the allowed visit window are to be documented. | Table 1<br>Table 3<br>Section 6.2.2.7 |
| 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. 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:<br>Section 6.2.3.12 (Other Safety Laboratory Evaluations)<br>Section 6.2.5 (General Information Regarding Laboratory Sampling and Results). | Table 4<br>Section 6.2.3.12<br>Section 6.2.5 |
| 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 |

| Summary of Changes | | |
|---|---|---|
| **Amendment Number:** 4.0 (Version 5.0) | **Amendment Date:** 15 Oct 2020 | **Global/Country/Site-Specific:** Global |
| **Description of Change** | | **Section(s) Affected by Change** |
| The primary objective and endpoints were updated to include factor IX activity after 12 months (52 weeks) of AMT-061 treatment and the non-inferiority assessment of AMT-061 during the post-treatment follow-up compared to standard of care continuous routine factor IX prophylaxis treatment during the lead-in period, which were both previously secondary objectives/endpoints. Text references to the objectives throughout the protocol were updated as needed.<br>CCI [redacted]<br>Safety endpoints were updated for alignment and completeness. | | Synopsis<br>Section 2<br>Section 3.3<br>Section 9.9 |
| 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 post-treatment follow-up phase, long-term follow-up phase, and PROBE and MSKUS sub-study. | | Synopsis |
| Clarification that paper diaries are to be used during the long term follow 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. | | Synopsis<br>Table 3<br>Section 6.1.5<br>Section 6.2.2.1, 6.2.2.2 |
| Addition of a visit window for the PROBE questionnaire to be completed for Visit F15 (Month 6) and Visit F-Final (Month 12) and during the long-term follow-up period if those visits are impacted by COVID-19. | | Synopsis<br>Section 6.2.2.6 |
| Addition of a visit window for MSKUS to be completed for Visit F15 (Month 6) and Visit F-Final (Month 12) and during the long-term follow-up period if those visits are impacted by COVID-19. | | Synopsis<br>Section 6.2.2.7 |

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| 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 as agreed between the Investigator and subject following the notification of a first negative result on blood and/or semen. | Table 2, Table 4<br>Section 6.2.3.10 |
| Section 1.6 "Accomodations 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 patients 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<br>Section 6.2.2.1 |
| Additional clarification and recommendations on transaminase elevation management was added including discussions on steroid tapering, considerations with prolonged high dose steroid use, and considerations with potential and confirmed COVID-19 infections. | Section 5.6.4 |
| Addition of a visit window for CCI [redacted] to be completed for Visit F15 (Month 6) and Visit F-Final (Month 12) and during the long-term follow-up period if those visits are impacted by COVID-19. | Section 6.2.2.4 |
| Addition of a visit window for abdominal ultrasounds to be completed for Visit F-Final (Month 12) and during the long-term follow-up period if those visits are impacted by COVID-19. | Section 6.2.3.4 |
| Removal of use of international criteria for assessment of laboratory value abnormalities. | Section 7.3.1 |
| 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 protocol deviations includes those related to COVID-19. | Section 9.5 |

| **Summary of Changes** | | |
|---|---|---|
| **Amendment Number:** 3.0 (Version 4.0) | **Amendment Date:** 30-Aug-2019 | **Global/Country/Site-Specific:** Global |
| **Description of Change** | | **Section(s) Affected by Change** |
| Contact details for Medpace Clinical Safety were updated. | | Emergency Contact Information |
| Under Trial Period (Planning), the timing of last subject last visit was updated to Q2 2025. | | Synopsis |
| Details on sponsor-preapproved alternatives that may be used to collect liver fibrosis scores were added. | | Synopsis<br>Table 1<br>Section 6.1.1, 6.1.2 |
| An abdominal ultrasound to screen for liver malignancy was added at Visit L-Final at the latest (to establish baseline), Visit F-Final, and Visits LTF2, LTF4, LTF6, and LTF8.<br>Section 6.2.3.4 Abdominal Ultrasound was added. | | Synopsis<br>Table 1 and Table 3<br>Section 6.1.2 and 6.1.4<br>Section 6.2.3.4 |
| Vital signs (blood pressure, pulse, body temperature) was removed from the list of safety endpoints. | | Synopsis<br>Section 3.3.2 |
| Exclusion criterion #3 was updated to allow subjects with total bilirubin >2 times upper normal limit if this elevation is caused by Gilbert disease.<br>Assessment of exclusion criterion #5 was updated to occur at screening (Visit S). Additionally, positivity for hepatitis B extracellular antigen (HBeAg) was removed as an additional criterion to Hepatitis B or C infection. | | Synopsis<br>Section 4.2 |
| Description of study endpoints and statisticial analyses were updated to align with the current plans described in the SAP, including addition of sub-study endpoints and clarification of the study populations to be analyzed. | | Synopsis<br>Section 3.3.2, 3.3.3<br>Section 9.4, 9.5, 9.6, 9.7, 9.9 |
| It was clarified that blood sampling occurs prior to Factor IX administration, and the requirement that this occurs at the clinic was removed. | | Table 1, Table 2, Table 3, Table 4<br>Section 6.2.2.3 |
| The visit window for Visit S was updated to -28 days | | Table 1 and Table 2 |
| The visit window for Visit L-Final was updated to -28 days (±7 days) from Visit D and it was specified that the time period between Visit LX and Visit L-Final can be less than 2 months as long as the total lead-in period is a minimum of 26 weeks. | | Table 1 and Table 2<br>Section 3.1<br>Section 6.1.2 |

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|---|---|
| For recommended alcohol use, it was recommended that the daily alcohol limit in grams be adhered to most strictly during the first 12 weeks post-IMP and that the importance of abstaining from binge-drinking be reinforced at every visit. | Table 1<br>Section 6.1.1 |
| For subjects travelling to a different site for Visit D, it was clarified that they would receive their treatment card at the L-Final visit and instructed to bring it with them to Visit D. Subjects travelling for Visit D may provide their semen sample at Visit L-Final. | Table 1<br>Section 3.1<br>Section 6.1.3.3<br>Section 10.3 |
| The end of the clinical trial was defined as the point in time the last patient has completed the long-term follow-up observation period of 5 years after administration of the investigational medicinal product. | Section 3.1 |
| Text was added explaining that Subjects who withdraw from the trial after enrolling in the Lead-in Phase but who are not dosed will be required to return their e-diary device and will not be requested to complete any final evaluations. | Section 4.5 |
| It was clarified that each vial of AMT-061 will contain an extractable volume of at least 10 mL. | Section 5.2 |
| It was clarified that subjects can receive their dose of continuous routine factor IX on the day of AMT-061 dosing. | Section 5.6.2<br>Section 6.1.3.3 |
| Section 5.6.3 Guidelines for Use of Factor IX for Patients Undergoing Major Surgery was added. | Section 5.6.3 |
| It was clarified that visits during the post-treatment follow-up period (from Week 1 up until Week 52) that do not include physical examination and/or CCI [redacted] may be performed at the subject's home by an appropriately qualified and trained nurse, with agreement from the Investigator and Sponsor. | Section 6.1.4 |
| The details on type of medical history data related to hemophilia B recorded were updated. | Section 6.2.1.4 |
| It was clarified that for subjects who are not able to enter the details of their factor IX replacement therapy into the e-diary, the site can enter the information in the CRF as long as the subject provides sufficient source documentation. | Section 6.2.2.1 |
| It was clarified that for subjects who are not able to enter the details of their bleeding episodes into the e-diary, the site can enter the information in the CRF as long as the subject provides sufficient source documentation. | Section 6.2.2.2 |

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| The description of CCI was updated, to include mention that this includes an assessment of the time sitting. | Section 6.2.2.4 |
| It was specified that the methodology for MSKUS plus scoring of MSKUS will be performed according to the J.A.D.E. protocol. | Section 6.2.2.7 |
| It was clarified that the Investigator will be provided with those laboratory results needed for treatment decision-making (i.e., laboratory results that inform safety monitoring or management of an individual subject at a site). The Investigator will receive chemistry, hematology, serology, coagulation, and gene sequencing results at regular intervals for review and sign-off. Once there have been three consecutive negative results for vector genome detection (in blood and semen), the Investigator will be informed that these samples no longer need to be collected. | Section 6.2.5 |
| The approximately volume of blood to be drawn from each subject during the trial was lowered to 2370 mL. | Section 6.2.6 |
| Pharmacovigilance Department was updated to Medpace Clinical Safety. | Section 7.5 |
| It was clarified that for clinical data, domains will be mapped to the current version of CDISC SDTM at the time of mapping. | Section 8.3.1 |
| Storage, secondary packaging, shipping, and EU Qualified Person (QP) release of IMP was added to the list of services vendors are engaged for in this trial | Section 11.4 |
| Laboratory details for testing of PBMC isolation for AAV5-capsid specific T-cell response, and of NABs, were updated. | Section 13.1 |
| Appendix 13.2 Drug Induced Liver Injury Dataset was removed and reference to the source added in the protocol body. | Section 5.6.2<br>Section 13.2 (previous) |

| **Summary of Changes** | | |
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| **Amendment Number:**<br>2.0 (Version 3.0) | **Amendment Date:**<br>07 Dec 2018 | **Global/Country/Site-Specific:**<br>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 |
| 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 |
| The planned number of sites was increased to approximately 50. | | Synopsis<br>Section 3.5 |
| The dose of $2 \times 10^{13}$ gc/kg AMT-061 will be the dose used in this study, as confirmed in the interim results from the CT-AMT-061-01 dose confirmation study. Text throughout the protocol was updated to reflect this. | | Synopsis<br>Section 1.3.2.3<br>Section 1.4<br>Section 3.2<br>Section 9.12 |
| Interim analysis results from the Phase IIb study CT-AMT-061-01 was added and sections updated to highlight CT-AMT-061-01 data and focus on AMT-061. | | Synopsis<br>Section 1.3<br>Section 1.4<br>Section 1.5 |
| FibroScan™ was added as an assessment at screening to deterimine if a subject has liver fibrosis and steatosis. A screening FibroScan assessment is not needed if either a FibroScan assessment has been performed in the year prior to screening for which CAP scores are available or if a liver biopsy has been performed within the 2 years prior to the screening for which fibrosis grade is documented. | | Synopsis<br>Trial Schedules<br>Section 6.1.1 |
| A discussion between the Investigator or designee with the subject on the importance of a healthy liver before and after receiving a liver directed gene therapy, and factors that might impact liver health was added as a continuous assessment from screening onwards. | | Synopsis<br>Trial Schedules<br>Section 6.1.4 |

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| It was clarified that the lead-in phase will last for a minimum of 6 months ending at or before Visit D. Subjects will remain in the lead-in phase until a minimum of 6 months of lead-in data have been collected and it is confirmed that the subjects still meet all eligibility criteria, with the final visist occuring approximately four weeks (6 weeks maximum) prior to the planned data of IMP dose administration (Visit D). | Synopsis<br>Trial Schedules<br>Section 3.1<br>Section 6.1.2 |
| Criteria for IMP dose administration was removed as the interim results of the dose confirmation trial are now available. | Synopsis<br>Figure 2<br>Trial Schedules<br>Section 3.1<br>Section 6.1.2 |
| It was clarified that at Visit L-Final, re-evaluation of subject's eligibility will be based on assessments during both the lead-in period and this visit. | Synopsis<br>Section 6.1.2 |
| It was clarified that the wash out period will be 3 days for regular-acting factor IX products and 10 days for extended half-life factor IX products. | Synopsis<br>Trial Schedules<br>Section 3.1 |
| It was specified that if the ICF is revised during the lead-in pahse with lead-in phase with important new information that must be shared with the study subjects, the amended ICF will be presented, as required by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), for review and consideration by the subject, and signed re-consent is to be obtained prior to IMP dose administration. | Synopsis<br>Section 3.1<br>Section 6.1.2<br>Section 10.3.1 |
| During the post-treatment follow-up, visits that do not include physical examination or CCI [redacted] may be performed at the subject's home by an appropriately qualified and trained nurse, with agreement from the investigator and the Sponsor. Each home nursing visit is expected to be supplemented by a phone call from the Investigator or designee to the subject to discuss AEs, concomitant medications, and e-diary compliance. | Synopsis<br>Trial Schedules<br>Section 6.1.4 |
| 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<br>Section 5.6.2<br>Section 6.1.4 |

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| 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 5.6.3 was created as the section for Guidelines for ALT Elevations. | Synopsis<br>Section 5.6.3 |
| It was clarified that assessment of blood pressure, pulse, and body temperature was assessment of vital signs. | Synopsis<br>Trial Schedules<br>Section 3.3.2<br>Section 6.2.3.2 |
| Assessment of Exclusion Criterion #5 was clarified to occur at Visit L-Final only. Exclusion criteria 5 and 6 were combined into a single criterion.<br>Exclusion Criterion #9 was updated to include advanced liver fibrosis (suggestive of or equal to METAVIR Stage 3 disease; a FibroScan™ score of >9 kPa was considered equivalent).<br>Exclusion Criterion #11 related to known history of allergy to corticosteroids was added. | Synopsis<br>Section 4.2 |
| Descriptions of statistical analyses were expanded, including update of the Full Analysis Set and Per Protocol population definitions, specification that the primary analysis will be performed using a repeated measures linear mixed model, adjusting the order the secondary endpoints will be analyzed in, CCI [redacted] | Synopsis<br>Section 2.2<br>Section 3.3.2<br>Section 9 |
| Alpha-fetoprotein was added as an assessment (measured at Visit L-Final, Visit F15, Visit F-Final, and all LTF visits) and as a safety endpoint. | Synopsis<br>Trial Schedules<br>Section 3.3.2<br>Table 8<br>Section 9.10 |
| Definition of gc was updated to genome copies from gene copies in the List of Abbreviations. | List of Abbreviations |
| 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<br>Section 6.2.2.3 |

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| Dose of routine factor IX and its associated footnote was removed from the Trial Schedules. | Trial Schedules |
| It was clarified that CCI being completed by the subject prior to other assessments are performed was a recommendation. | Trial Schedules<br>Section 6.1.2<br>Section 6.1.4<br>Section 6.1.5<br>Section 6.2.2.4<br>Section 6.2.2.6 |
| For the optional musculoskeletal ultrasound sub-study, it was clarified that if it is not possible to obtain the musculoskeletal ultrasound at screening (Visit S), it is allowed to obtain this first musculoskeletal ultrasound at a later time point (preferably as soon as possible thereafter during one of the lead-in visits). | Trial Schedules<br>Section 6.2.2.7 |
| Details for the second and last reference listed in Table 5 were updated. | Table 5 |
| The title for Section 1.3.2.1 was updated to “Preliminary Clinical Evidence for Efficacy and Safety of Components of AMT-061”, instead of AMT-060. | Section 1.3.2.1 |
| Preliminary results from a Phase I/II trial of an AAV5-B-domain-deleted factor VIII construct was updated with initial results.<br>Preliminary results from a single study showing sustained factor IX expression driven by a gene cassette encoding a Padua variant factor IX was updated with initial results. | Section 1.3.2.1 |
| In discussion of the results from trial AMT-060-01, clarification was made regarding how many subjects shifted to a moderate or severely moderate hemophilia phenotype during the study. | Section 1.3.2.2 |
| Clarification that transaminase increase alone may warrant the initiation of a corticosteroid was made in Section 1.5.3, for consistency with Section 5.6.2. | Section 1.5.3 |
| The study population was clarified to include subjects with more than 150 exposure days to a factor IX product in Section 1.5.8 for consistency with the inclusion/exclusion criteria. | Section 1.5.8 |
| It was clarified in Section 1.5.9 that subjects will be administered a dose of their usual factor IX product/dose for their factor IX recovery assessment at Visit L-Final, not also at the screening visit. | Section 1.5.9 |

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| It was clarified that during the lead-in phase, an email communication is acceptable as an alternative to a follow-up phone call if the subject cannot be reached, though reasonable efforts for a phone call should be made. | Section 3.1<br>Section 6.1.2 |
| Clarifications were added surrounding concomitant medication/therapy. | Section 5.6.2 |
| It was clarified that factor IX gene sequence analyses will be performed for all subjects that provide consent, even those that already have factor IX gene mutation information. | Section 6.1.1<br>Section 6.2.1.6 |
| 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 |
| Collection at screening of on-demand factor IX replacement therapy at trial entry was removed from the list of medical history data related to hemophilia B being collected. | Section 6.2.1.4 |
| Definitions for joint and muscle bleeds were removed.<br>The definition for persistent bleed was added. | Section 6.2.2.2 |
| It was clarified that the short form of the CCI [redacted] will be used in this study. | Section 6.2.2.4 |

| **Summary of Changes** | | |
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| **Amendment Number:**<br>1.0 | **Amendment Date:**<br>29 Mar 2018 | **Global/Country/Site-Specific:**<br>Global |
| **Description of Change** | | **Section(s) Affected by Change** |
| The title of the trial was updated, changing "an serotype" to "a serotype."<br>Current: Phase III, open-label, single-dose, multi-center multinational trial investigating **a** 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<br>Previous: Phase III, open-label, single-dose, multi-center multinational trial investigating **an** 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 | | Title |
| Administrative change to the identity of the study monitor. | | Title Page |
| Inclusion Criterion #4 updated.<br>Current: >150 previous exposure days of treatment with FIX protein.<br>Previous: >20 previous exposure days of treatment with FIX protein. | | Synopsis<br>Section 4.1 |
| Specification that Exclusion Criterion #2 will be based on local laboratory results.<br>Specification that Exclusion Criteria #3, 4, 5, and 8 will be based on central laboratory results. | | Synopsis<br>Section 4.2 |
| Rewording of visit descriptions to improve clarity of assessments and their order. | | Synopsis<br>Section 3.1<br>Section 6.1 |
| 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<br>Section 6.1.5<br>Section 6.2.2.1<br>Section 6.2.2.2 |

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| Update to language stating that Investigators will assess each bleeding episode, not adjudicate each bleeding episode. | Synopsis<br>Schedule of Events Table 1<br>Section 6.1.2<br>Section 6.1.6<br>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<br>Section 6.2.2.3<br>Section 9.9 |
| Removal of the CCI assessment at Visit L3. | Schedule of Events Table 1 |
| Adjustment of timing of physical examination assessments. Physcial examination assessments will occur at screening (Visit S), Visit L-Final, Visit D (pre-IMP), Visits F1, F2, F4, F6, F12, f13, F15, F17, F19, F-Final, LTF1, LTF2, LTF3, LTF4, LTF6, and LTF8. | Schedule of Events Table 1<br>Section 6.1.4<br>Section 6.1.5<br>Section 6.2.3.3 |
| Addition of a chromogenic assay for FIX activity with assesments at Visits L1 to L-Final, Visit D, pre-IMP, Visits F1 to F-Final, and Visits LTF1 to LTF8. | Schedule of Events Table 2 and Table 4<br>Section 6.2.2.3<br>Section 6.2.3.6 |
| Clarification of the duration of trial participation.<br>Current: The overall trial participation will be approximately five and a half years.<br>Previous: The overall trial participation will be a minimum of five and a half years. | Section 3.1 |
| Addition of “Withdrawal by principal Investigator” as a possible reason for discontinuation. | Section 4.5.1 |
| The fill volume of IMP vials provided was clarified to be approximately10 mL. | Section 5.2 and Table 7 |
| Update of the names of the 2 PROBE forms.<br>Addition of a description of both the Short Form and the Follow-up Form.<br>Clarification that the Follow-up Form will be completed at Visit L3. | Section 6.2.2.6 |
| Clarification that musculoskeletal ultrasounds will be centrally analyzed and results assessed by subject over the course of the study. Full details can be found in the Musculoskeletal Ultrasound Manual. | Section 6.2.2.7 |
| Removal of vendor information for vector genome detection.<br>Clarification of changes to the sampling schedule. | Section 6.2.3.9 |

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| Clarification that local laboratory results should be provided to the Investigator as soon as possible. | Section 6.2.3.11 |
| The volume of blood to be drawn from each subject was updated due to the addition of the chromogenic assay for FIX activity.<br>Current:<br>• Approximately 2450 mL blood (excluding additional visits) total.<br>• Maximum of 120 mL per visit and maximum of 110 mL per additional visit.<br>• Approximately 2000 mL between screening and the end of the post-treatment follow-up<br>• Approximately 450 mL during the long-term follow-up.<br>Previous:<br>• Approximately 2250 mL blood (excluding additional visits) total.<br>• Maximum of 110 mL per visit and maximum of 100 mL per additional visit.<br>• Approximately 1850 mL between screening and the end of the post-treatment follow-up<br>• Approximately 400 mL during the long-term follow-up. | Section 6.2.6 |

# Signature Page

## CT-AMT-061-02 - Protocol Amendment - AMD 7 CSP_V8_2022_02_07

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| Approved-Clinical Development Physician Approval | |
| PPD | PPD 16:23:02 |
| Approved-PPD Approval | |
| PPD | PPD 16:31:51 |
| Approved-PPD Approval | |
| PPD | PPD 19:37:27 |
| Approved-Clinical Safety Physician Approval | |



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