15 December 2022
EMA/46569/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Hemgenix

International non-proprietary name: etranacogene dezaparvovec

Procedure No. EMEA/H/C/004827/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Table of contents

List of abbreviations

AAV	Adeno-associated virus
AAV2	Adeno-associated virus serotype 2
AAV5	Adeno-associated virus serotype 5
AE	Adverse event
AFP	Alpha-fetoprotein
ALT	Alanine aminotransferase
AMT-060	Predecessor of etranacogene dezaparvovec
AMT-061	Etranacogene dezaparvovec, AAV5-hFIXco-Padua
AST	Aspartate aminotransferase
Bac	Baculovirus
BEVS	Baculovirus expression vector system
BMI	Body mass index
bp	base pair
Cap	Capsid gene
cDNA	Complementary DNA
CoA	Certificate of analysis
CPP	Critical process parameter
CPV	Continued process verification
CQA	Critical quality attribute
CSR	Clincal study report
DP	Drug product
DS	Drug substance
ELISA	Enzyme-linked immunosorbent assay
EHL	Extended half-life
FIX	Clotting factor IX
FIX-Padua	Human coagulation factor IX variant R338L
gc	Genome copies
HCP	Host cell protein
hFIX	Human factor IX
hFIXco-Padua	Codon optimised coding sequence for the human Factor IX variant R338L
hFIX-Padua	Human coagulation factor IX variant R338L
HMWS	High molecular weight species
IFNγ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
INN	International non-proprietary name
ip	Infectious particles
IPC	In process control
IPS	In process specification
IRS	Initial reference standard
ISS	Integrated summary of dafety
ITR	Inverted terminal repeat
iu	Infectious units
IU/ml	International units per millilitre
IV	Intravenous
KPA	Key process attribute
KPP	Key process parameter
LLOQ	Lower limit of quantification
LOD	Limit of detection
MCB	Master cell bank
MCP-1	Monocyte chemoattractant protein-1
MSV	Master seed virus

MW	Molecular weight
N/A	Not applicable
nAb	Neutralising antibodies
NHP	Non-human primate
NOR	Normal operating range
OOS	Out of specification
PAR	Proven acceptable range
PBS	Phosphate buffered saline
PCS	Process control strategy
PRS	Primary reference standard
PS-20	Polysorbate-20
PT	Preferred term
PV/PPQ	Process validation / process performance qualification
QP	Qualified person
qPCR	Quantitative polymerase chain reaction
rAAV	Recombinant adeno-associated virus
rcAAV	Replication competent adeno-associated virus
Rep	Replicase gene
RT	Room temperature
RU	Relative units
SAE	Serious adverse event
SAP	Statistical analysis plan
SHL	Standard half-life
SOC	System organ class
SRS	Secondary reference standard
TEAE	Treatment-emergent adverse event
tp	Total particles
TSE	Transmissible spongiform encephalopathy
ULOQ	Upper limit of quantification
w/v	Weight per volume
WCB	Working cell bank
WGS	Whole genome sequencing
wt	Wild type

1. Background information on the procedure

1.1. Submission of the dossier

The applicant CSL Behring GmbH submitted on 7 March 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Hemgenix, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 21 April 2017.

Hemgenix, was designated as an orphan medicinal product EU/3/18/1999 on 21 March 2018 in the following condition: Treatment of haemophilia B.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Hemgenix as an orphan medicinal product in the approved indications. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/Hemgenix

The applicant applied for the following indication:

Treatment of adults with Haemophilia B (congenital Factor IX deficiency) and with a preexisting neutralising anti-AAV5 antibody titre below 1:700 to reduce the frequency of bleeding episodes and the need for Factor IX replacement therapy who:

- *currently use Factor IX prophylaxis therapy,*
- *or have current or historical life-threatening haemorrhage or repeated, serious spontaneous bleeding episodes.*

1.2. Legal basis and dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that etranacogene dezaparvovec was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies.

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0380/2022 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0380/2022 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant submitted a critical report addressing the possible similarity with authorised

orphan medicinal products.

1.5. Applicant's request(s) for consideration

1.5.1. Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

1.5.2. Conditional marketing authorisation

During the assessment, the applicant requested consideration of its application for a conditional marketing authorisation in accordance with Article 14-a of the above-mentioned Regulation.

1.5.3. New active substance status

The applicant requested the active substance etranacogene dezaparvovec contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.5.4. Scientific recommendation on classification

The applicant CSL Behring GmbH submitted on 7 March 2022 an application for scientific recommendation on classification to the European Medicines Agency (EMA) for Hemgenix, which was designated as an advanced therapy medicinal product on 21 April 2017.

1.6. PRIME

Hemgenix was granted eligibility to PRIME on 21 April 2017 in the following indication: treatment of severe haemophilia B.

Eligibility to PRIME was granted at the time in view of the following:

- the unmet need may be acknowledged in particular on the basis of breakthrough bleeds and the development of bleeding sequelae such as haemophilic arthropathy;

- the potential to address the need can be accepted on the basis of preliminary clinical observations in patients with FIX activity ≤ 2% of normal;

- in the study presented a single IV administration resulted in a sustained increase in factor IX activity, allowing for interruption of prophylactic treatment for the majority of treated patients.

Upon granting of eligibility to PRIME, Ilona G. Reischl was appointed by the CAT as rapporteur.

A kick-off meeting was held on 03 October 2017. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

- CHMP scientific advice on quality and non-clinical aspects including the overall comparability strategy to support changes in manufacturing and the formulation.

- CHMP scientific advice on clinical development including the phase IIb and pivotal phase 3 study designs to support the future MAA, and advice on the paediatric studies related to the PIP.

- An orphan designation application was recommended to cover the current development product AMT-061.

1.7. Protocol assistance

The applicant received the following protocol assistance on the development relevant for the indication subject to the present application:

Date	**Reference**	**SAWP co-ordinators**
16 February 2012	EMEA/H/SA/2271/1/2012/ADT/SME/I	Gopalan Narayanan, Caroline Auriche
20 September 2012	EMEA/H/SA/2271/2/2012/PA/SME/ADT/II	Jan Mueller-Berghaus, Thomas Lang
23 June 2016	EMEA/H/SA/2271/3/2016/PA/SME/ADT/III	Jan Mueller-Berghaus, Olli Tenhunen
22 February 2018	EMEA/H/SA/3720/1/2017/SME/ADT/PR/III	Andrea Laslop, Olli Tenhunen
22 February 2018	EMEA/H/SA/3720/2/2018/SME/ADT/PR/I	Olli Tenhunen, Fernando de Andrés Trelles
31 May 2018	EMEA/H/SA/3720/3/2018/SME/ADT/PR/HTA/II	Andrea Laslop, Peter Mol
19 September 2019	EMEA/H/SA/3720/5/2019/PA/SME/ADT/PR/II	Jan Mueller-Berghaus, Andrea Laslop
26 March 2020	EMEA/H/SA/3720/2/FU/2020/PA/SME/ADT/PR/I	Carin Bergquist, Fernando de Andrés Trelles

The protocol assistance pertained to the following quality, non-clinical, and clinical development aspects:

- The comparability strategy to address changes in the manufacturing process for AMT-060 (predecessor of AMT-061) to be employed for clinical phase III and commercial vector production. Proposal to adjust the manufacturing process in order to improve process and product consistency.

  The comparability strategy to address changes in the manufacturing process to be employed for clinical phase III and commercial scale for AMT-061. The strategy for qualification and characterisation.

- Acceptability of the proposed process validation plan for AMT-061. The proposed plan to support the change to the drug product specification and testing strategy. Design of a toxicity and biodistribution study in C57BLl/6 mice with AMT-060, including the effects of a corticoid regimen. Risk assessment of inadvertent germ line transmission of AMT-060. Sufficiency of the proposed non-clinical development plan for AMT-060 to support a MAA.

  Sufficiency of the nonclinical GLP study (NR-061-17-001) conducted in cynomolgus macaques with AMT-061 and AMT-060 to support comparability with AMT-060 with respect to biodistribution

and safety. Sufficiency of the GLP nonclinical study in male mice with AMT-061 and AMT-060 to evaluate comparable activity and safety of the two products with and without polysorbate 20. Adequacy of the totality of nonclinical data to support the pivotal study of AMT-061 and MAA.

- Design of a FIM Phase I/II dose escalation study with AMT-060, including starting dose and dose escalation scheme, the use of prednisone as a short rescue treatment in case of hepatic inflammatory signs/symptoms, and approach to evaluate the clinical benefit.

  Design of the proposed open-label, single-dose, single-arm Phase 3 study for AMT 061, and sufficiency of the single pivotal study, together with supportive data from the Phase 1/2 study with AMT-060, to support MAA. The development plan for AAV5-NAB. The proposed approach to substantiate longer-term efficacy and safety claims.

  Design of a drug-specific core registry and five separate registry substudies (1. Pre-existing anti-AAV5 neutralizing antibodies (NABs), 2. Liver Health, 3. Clinical Benefit, 4. Quality of Life, 5. Surgical outcomes) to provide long-term efficacy and safety FU of clinical trial and commercially treated patients.

1.8. Steps taken for the assessment of the product

The CAT Rapporteur and Co-Rapporteur appointed by the CHMP were:

CAT Rapporteur: Ilona G. Reischl CAT Co-Rapporteur: Heli Suila

The appointed CAT co-rapporteur had no such prominent role in protocol assistance relevant for the indication subject to the present application.

The application was received by the EMA on	7 March 2022
Accelerated Assessment procedure was agreed-upon by CAT and CHMP on	24 September 2021
The procedure started on	24 March 2022
The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	13 June 2022
The CAT Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	13 June 2022
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	27 June 2022
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT during the meeting on	7 July 2022
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on The assessment timetable was reverted back from accelerated to standard timelines.	15 July 2022
The applicant submitted the responses to the CAT consolidated List of Questions on	8 August 2022
The CAT Rapporteur circulated the Joint Assessment Report on the	14 September 2022

responses to the List of Questions to all CAT and CHMP members on	
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	29 September 2022
The CAT agreed on a list of outstanding issues in writing to be sent to the applicant on	7 October 2022
The applicant submitted the responses to the CAT List of Outstanding Issues on	8 November 2022
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT, PRAC and CHMP members on	24 November 2022
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Hemgenix on	9 December 2022
The CAT adopted a report on similarity of Hemgenix with Alprolix and Idelvion on	9 December 2022
Furthermore, the CAT adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product	9 December 2022
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Hemgenix on	15 December 2022
The CHMP adopted a report on similarity of Hemgenix with Alprolix and Idelvion on	15 December 2022
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product	15 December 2022

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The claimed indication of Hemgenix (etranacogene dezaparvovec) is the treatment of adults with haemophilia B (congenital factor IX deficiency) and with a pre-existing neutralising anti-AAV5 antibody titre below 1:700 to reduce the frequency of bleeding episodes and the need for Factor IX replacement therapy who:

• currently use factor IX prophylaxis therapy,

or

• have current or historical life-threatening haemorrhage or repeated, serious spontaneous bleeding episodes.

2.1.2. Epidemiology

Congenital haemophilia B is an X-linked inherited bleeding disorder, almost exclusively in males, characterised by an increased bleeding tendency due to either a partial or complete deficiency of the essential blood coagulation FIX. The deficiency is the result of mutations of the respective clotting factor genes. Approximately 1 in 20,000 to 50,000 live male newborns have haemophilia.

Based on historical classification using functional FIX levels, approximately one-third of individuals have a severe disorder characterised by functional FIX levels < 1% of normal, approximately one-third of individuals have moderate haemophilia B, with 1 to 5%, and approximately one-third of individuals have mild haemophilia B with > 5 to < 40% of normal FIX levels [White, et al, 2001].

However, individuals may exhibit a severe bleeding phenotype irrespective of their FIX level, including individuals with current or historical repeated spontaneous bleeding episodes (which may include joint or life-threatening haemorrhage), established joint damage due to haemarthroses, and / or the current use of factor IX continuous prophylaxis.

2.1.3. Aetiology and pathogenesis

Haemophilia B is an inherited bleeding disorder characterised by an increased bleeding tendency due to either a partial or complete deficiency in the activity of the essential blood coagulation factor IX. Haemophilia B is an X-linked, recessive condition, and occurs primarily in males. Females are typically carriers with a mild or absent bleeding phenotype.

2.1.4. Clinical presentation

Intra-articular and intramuscular bleeding is a major clinical manifestation of the disease. Bleeding most commonly occurs in the knees, elbows, and ankles. The pathogenesis of haemophilic arthropathy is multifactorial, with changes occurring in the synovium, bone, cartilage, and blood vessels. Recurrent joint bleeding causes synovial proliferation and inflammation (haemophilic synovitis) that contribute to end-stage degeneration (haemophilic arthropathy); with pain and limitation of motion severely affecting patients' quality of life (QoL) [Knobe and Berntorp, 2011]. The severity of bleeding manifestations generally correlates with the degree of the clotting factor deficiency. Severe forms become apparent early in life [Srivastata et al, 2020].

2.1.5. Management

There is no cure for haemophilia B. The primary goals of haemophilia B therapy are the prevention of bleeding episodes, rapid and definitive treatment of bleeding episodes (breakthrough bleeding episodes) that occur even while on a regular prophylactic regimen and provision of adequate haemostasis during surgery and emergencies. Currently, these goals are essentially met for haemophilia B subjects by intravenous (IV) injections of commercially available recombinant- or plasma-derived FIX products, either at the time of a bleeding episode (on-demand) or by regular infusions up to several times a week (prophylactically). The recent approvals of extended half-life FIX products allow for reduced frequency of factor administration (once every 7 to 14 days) and maintenance of a higher FIX trough level.

The current treatment options for haemophilia B have several limitations. Treatment with prophylactic regular IV injections of FIX 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 FIX infusion result 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 morbidity and mortality rate.

There is also a risk of developing neutralizing antibodies (nAbs) against the administered FIX. 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 haemophilia can have a substantial effect on mental wellbeing, particularly among young people and signs of major depressive disorder are not uncommon. The economic burden for the society is significant.

2.2. About the product

Etranacogene dezaparvovec is a gene therapy medicinal product that employs a non-replicating, recombinant adeno-associated virus-based vector serotype 5 (AAV5) containing a codon-optimised coding DNA sequence for the human coagulation Factor IX variant R338L (FIX-Padua) under the control of a liver-specific promoter (LP1).

Etranacogene dezaparvovec is produced using recombinant baculovirus technology.

Etranacogene dezaparvovec is delivered by a single intravenous dose and is designed to achieve prolonged expression of active human FIX in the plasma, synthesised from vector-transduced liver tissue.

2.3. Type of application and aspects on development

The CHMP and CAT agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on Hemgenix ability to induce endogenous factor IX expression and to provide patients with a more physiological mode of factor replacement without the need for a chronic infusion/ injection therapy. This ultimately could represent a curative option for patients besides the current substitution treatment.

However, during assessment the CAT and CHMP concluded that it was no longer appropriate to maintain accelerated assessment, in view of the outstanding Major Objections. The timetable was switched to standard.

During the assessment, the applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14-a of the above-mentioned Regulation, based on the following criteria:

- The benefit-risk balance is positive.
- It is likely that the applicant will be able to provide comprehensive data. By way of specific obligations, the applicant committed to provide the final CSRs for the ongoing studies CT-AMT-061-01 and CT-AMT-061-02 for the entire study duration (ie, 5 years) and will provide the requested 1 year interim analysis report with the first 50 patients enrolled in Study CSL222_4001.
- Unmet medical needs will be addressed, as although satisfactory methods of treatment of the condition exist in the European Union, the applicant has provided sufficient justification for the assumption that Hemgenix will be of major therapeutic advantage to those affected by the condition. The product has a mechanism of action that offers the potential to reduce or eliminate the use of exogenous factor IX products currently authorised for the condition, and

the non-clinical data provided demonstrate significant improvement of circulating factor IX protein and activity levels in valid models of the condition.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. The applicant claimed that making Hemgenix available to patients while the collection of comprehensive efficacy and safety data will be ongoing is not expected to represent a risk to public health.

2.4. Quality aspects

2.4.1. Introduction

Hemgenix is a gene therapy medicinal product designed to introduce a copy of the human Factor IX (FIX) coding DNA sequence into hepatocytes to address the root cause of the haemophilia B disease. Hemgenix employs a non-replicating, recombinant adeno-associated virus-based vector serotype 5 (AAV5) containing a codon-optimised coding DNA sequence for the human coagulation Factor IX variant R338L (AAV5-hFIXco-Padua) under the control of a liver-specific promoter (LP1). The active substance etranacogene dezaparvovec is produced using recombinant baculovirus technology.

Hemgenix is presented as a concentrate for solution for infusion in single-use Type I glass vial containing 1×10^{13} genome copies (gc)/mL of etranacogene dezaparvovec. Each vial contains an extractable volume of 10 mL of concentrate for solution for infusion, containing a total of 1×10^{14} genome copies. The total number of vials in each pack corresponds to the dosing requirement for the individual patient, depending on the patient's body weight (recommended single dose of 2×10^{13} gc/kg body weight corresponding to 2 mL/kg body weight).

The active substance is formulated with sucrose, polysorbate-20, potassium chloride, potassium dihydrogen phosphate, sodium chloride, disodium phosphate, hydrochloric acid (for pH adjustment) and water for injections.

2.4.2. Active Substance

2.4.2.1. General information

Etranacogene dezaparvovec consists of a human FIXco-Padua (hFIXco-Padua) expression cassette, which is packaged within a recombinant AAV5, and administered by intravenous infusion into patients. The hFIXco-Padua expression cassette contains a codon-optimised coding DNA sequence encoding the R338L variant of human factor IX (FIX Padua) under the control of the liver-specific promoter LP1. In the liver, the vector transduces liver cells without genome integration, and vector DNA remains almost exclusively in episomal form.

The amino sequence of the hFIX-Padua protein is described. Structure and general properties are briefly but sufficiently described.

2.4.2.2. Manufacture, characterisation and process controls

Manufacturing process and process controls

Etranacogene dezaparvovec active substance is manufactured at uniQure, Inc., 113 Hartwell Avenue, Lexington, MA 02421-3125, USA. All sites involved the manufacture, controls and storage of the active substance comply with EU GMP.

Etranacogene dezaparvovec is produced using the Baculovius Expression Vector System (BEVS) that utilises an insect cell line derived from Spodoptera frugiperda Sf9 cells. The BEVS is composed of different recombinant baculoviruses, which serve to deliver the essential components to produce AAV containing the Padua variant of human factor IX gene (hFIX-Padua) in the producer cells. The etranacogene dezaparvovec active substance manufacturing process is divided in upstream and downstream process. The upstream process (USP) consists of production of etranacogene dezaparvovec by baculo-virus infection and purification using established biotechnology procedures.

The downstream process consists of a harvest and clarification step, followed by several purification steps ending with formulation and final fill.

The manufacturing process steps have been described in a high-level flow diagram as well as in more detailed flow charts and narratives for respective process steps.

Control of materials

The starting materials for manufacture of etranacogene dezaparvovec active substance consist of an insect cell line (derived from *Spodoptera frugiperda* Sf9 cells) and purified recombinant baculoviruses. The development and characterisation of the master cell bank (MCB) and working cell banks, as well as cells at the limit of age (CALs) are described. The produced seed viruses have been adequately characterised. The testing strategy of the seed viruses is compliant with Ph. Eur. 5.14.

Sufficient information on raw and starting materials used in the active substance manufacturing process has been submitted. Information on materials of biological origin and testing was sufficiently presented. The testing panel for future cell banks and virus seeds is acceptable taking into account the commitment of the applicant (**Recommendation 2**).

The stability programme for seed and cell banks is acceptable taking into account the commitment of the applicant (**Recommendation 3**).

Control of critical steps and intermediates

Control of critical steps and process material is achieved using process parameter controls and in-process testing. In-process testing is presented, the methods are described, and justification is provided for the defined limits and acceptance criteria. The selection of in-process tests is acceptable. Control of critical steps and intermediates is considered appropriate.

Process validation

The process validation (PV) strategy employs a 3-stage risk-based approach to the process validation lifecycle: Stage 1, Process Design, Stage 2, Process Performance Qualification (PPQ), and Stage 3, Continued Process Verification (CPV). PV/PPQ was performed to demonstrate that the process, when operated within the defined ranges, produces active substance that consistently meets all IPCs, IPSs, and release specifications.

The PV/PPQ was performed at commercial scale at uniQure's Lexington MA facility in the USA, which is a qualified facility for the manufacture of etranacogene dezaparvovec active substance. All equipment, utilities, and facilities were qualified prior to use in the PV/PPQ.

The information provided on the additional validation studies (media fill studies, filter validation, shipping validation) in general give no reason for concern, taking into account the commitment of the applicant (**Recommendation 8**). Levels of process and product-related impurities are controlled by active substance release specification. In addition, in the characterisation section of the dossier, data of clearance of process-related impurities has been provided for PPQ batches.

Throughout the lifecycle of the product, a statistical evaluation of the data will be performed to demonstrate that the process remains in a validated state of control (CPV). The applicant committed, as part of the ongoing CPV programme, to analyse data from all commercial batches at a regular frequency to ensure process performance and the process control strategy (PCS) are appropriate to ensure product quality (**Recommendation 4**).

Manufacturing process development

Manufacturing Process

Production of the active substance including site transfer and scale up activities has been adequately described. The comparability assessment was performed. The comparability studies include comparison of process performance, active substance and finished product batch release results and extended characterisation.

Process Control Strategy

Identification of critical quality attributes (CQAs) is based on regulatory requirements and a risk assessment for severity and uncertainty regarding safety/immunogenicity, potency/efficacy, and pharmacokinetics. Quality target product profile (QTPP), clinical/non clinical, structure/function, characterisation data, and prior knowledge (including scientific literature and platform knowledge from other programs) were utilised in the risk assessment. Routine testing (in-process or release) is performed for all CQAs either at active substance or finished product or both stages. Commercial PCS has been established based on a scientific and risk-based approach taking into consideration the process experience gained from clinical manufacturing and PV/PPQ activities. Process parameters (input) and process attributes (output) have been defined for each step of the manufacturing process and their criticality has been assessed based on the risk to impact CQAs.

The manufacture of clinical batches was controlled by action and alarm limits, which is considered the preliminary PCS. Prior to commencing PV/PPQ a review of the preliminary PCS was performed and proven acceptable ranges (PARs) and normal operating ranges (NORs) were assigned. Development of the PCS also included a late-stage failure modes and effects analysis (FMEA). Process parameter and process attribute ranges (PAR, NOR) are based either on manufacturing experience, data from previously manufactured batches, data from process development/characterisation studies, process parameter validation studies, vendor provided operational limits, or equipment and facility capabilities.

Overall, validation results showed that although NORs are very wide for some parameters, the data obtained with PV/PPQ batches are not that different.

Characterisation

Elucidation of structure

Etranacogene dezaparvovec active substance has been extensively characterised using different methods focused on capsid and vector identity and composition, biophysical characterisation, post-translational modifications (PTM), and biological activity.

Impurities

Impurities that are present or potentially present in the etranacogene dezaparvovec active substance were properly analysed. The levels of these impurities are below the assay limit of detection, or are low enough not to pose any risk to patient safety.

2.4.2.3. Specification

Specification

Specification for the active substance includes control of identity, purity and impurities, biological activity and other general tests.

The established release specifications cover most of the relevant characteristics of AAV vectors. They are in line with the *Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors* (EMEA/CHMP/GTWP/587488/2007 Rev. 1) and Ph. Eur. 5.14. *Gene transfer medicinal products for human use*. A recommendation has been included based on the applicant's commitment to developing and incorporating a release assay, proposing to introduce the method as a release test as a post-approval variation (**Recommendation 10**).

Analytical procedures

In general, the analytical methods used for release testing of the active substance are correctly described and validated taking into account the commitment of the applicant (**Recommendation 11**).

Batch analysis

Batch analysis is in general well presented. Batch-to-batch consistency is in general met.

Reference standards

The product derived primary reference standard (PRS) used for active substance batch release and stability testing is the same as for finished product testing. The in-house reference standards or materials used for process and product-related testing of impurities of active substance release and stability testing are well described and acceptable.

Container closure

The container closure system is sufficiently described. The container closure system is considered adequate for etranacogene dezaparvovec active substance.

2.4.2.4. Stability

Stability studies of active substance involve commercial and historical batches. The tested parameters include relevant quality attributes for potency, strength, purity and general quality attributes that are considered stability indicating. Overall, the proposed shelf life is acceptable taking into account the commitment of the applicant (**Recommendation 12**).

The study protocol for the stability data, based on long-term, accelerated and stressed conditions, is well designed. Photostability studies have been provided for the finished product.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Description of the product

Etranacogene dezaparvovec is a preservative-free, liquid formulation with a nominal concentration of $1x10^{13}$ genome copies (gc)/mL and is formulated in a sterile phosphate buffered saline (PBS) solution, pH 7.1 containing sucrose and polysorbate-20 (PS-20). For pH adjustment of the buffer, small amounts of hydrochloric acid compliant to compendial standards are used.

The excipients used in the etranacogene dezaparvovec finished product are qualitatively and quantitatively the same as in the active substance. The excipients comply with Ph. Eur. No novel excipient is used.

Hemgenix is supplied as a 10 mL solution (strength 1 x 10^{13} genome copies/mL) in a single-use Type I glass vial with stopper (chlorobutyl rubber), aluminium seal with a flip-off cap.

The total number of vials in each finished pack corresponds to the dosing requirement of the individual patient, depending on the body weight, and is provided on the package.

The excipients used in etranacogene dezaparvovec finished product comply with Ph. Eur. requirements, are commonly used in the manufacturing of parenteral pharmaceutical preparations and are thus considered acceptable.

No overage or overfill is included for the etranacogene dezaparvovec finished product.

The applicant identified the key physicochemical properties of the active substance that might affect finished product performance. Compatibility of active substance with the excipients of the finished product was demonstrated.

Pharmaceutical development

Details on the formulation development have been provided in the dossier and the formulation development of etranacogene dezaparvovec finished product is considered appropriately described.

The finished product container closure system consists of a depyrogenated 10 mL Type I glass vial, a stopper made of chlorobutyl rubber and an aluminium seal with a flip-off cap. Representative diagrams and information on the critical dimensions of the components are presented. The glass vial and rubber stoppers comply with USP and Ph. Eur. requirements.

All container closure components are received ready-to-use (sterile). Information on the sterilisation of the container closure is provided as outlined in EMA/CHMP/CVMP/QWP/850374/2015.

The suitability of the container closure system was adequately evaluated. The compatibility between etranacogene dezaparvovec finished product and the primary container closure materials as well as absence of adsorption of the AAV to the glass vial / stopper is demonstrated. Extractables and leachables have been appropriately addressed. Overall, the choice of container is considered appropriate, and based on the currently available data from the simulation studies and from the components' suppliers, the safety risk of extractables and leachables from manufacturing of etranacogene dezaparvovec finished product is considered acceptable taking into account the commitment of the applicant (**Recommendation 16**).

Microbiological attributes

No preservative has been added to the preparation. As terminal sterilisation is not appropriate for this type of product, sterility is assured during manufacture by aseptic practices and in-line filtration with sterilising grade filters. In-process testing includes a bioburden test at different steps of product manufacture. Final sterility testing is performed in compliance with USP<71> and Ph. Eur. 2.6.1. Container closure system components are tested for sterility by the supplier and prior to release for use in finished product manufacture.

The microbial ingress test is part of the aseptic process simulation.

Stability studies do not indicate any incompatibility issues.

2.4.3.2. Manufacture of the product and process controls

Manufacturers

All sites involved in manufacturing, controls and storage of the finished product operate in accordance with EU GMP.

The applicant requested an exemption from re-testing upon importation into the EU. The lack of detailed justification for such request was initially raised as a Major Objection. During the procedure, the applicant agreed to propose a staggered approach to transfer release testing to the EU within a defined timeframe. This is considered acceptable (**Recommendation 1**).

The proposed batch size and batch formula for minimum and maximum batch sizes are appropriately provided. The batch numbering system has been described.

Manufacturing process

The manufacturing process for etranacogene dezaparvovec finished product is described in the dossier and consists of 1) formulation buffer preparation, 2) thawing of the active substance, 3) sterile filtration and finished product compounding, 4) fill and finish, 5) visual inspection and bulk vial storage and 6) labelling, packaging and finished product storage.

In general, the description of the etranacogene dezaparvovec finished product manufacturing process is considered sufficient and acceptable. Hold times have been verified through process validation.

Each process parameter has been classified as key, critical or non-critical. Proven acceptable ranges or acceptance criteria have been established for each parameter. The applicant states that the classification of the manufacturing process variables was performed in accordance with internal policies and procedures. Acceptable ranges for process controls were determined using manufacturing data, process development reports, validation reports, stability study reports or vendor recommendations.

Process validation

The etranacogene dezaparvovec finished product manufacturing process was validated at the proposed commercial manufacturing site. Based on a risk-based approach three consecutive finished product batches meeting all PV/PPQ acceptance criteria were manufactured to validate the manufacturing process. The process validation strategy included a 3-stage risk-based approach to the process validation lifecycle; Stage 1 was a process design phase, process qualification was performed as Stage 2, and Stage 3 is a continued process verification programme. The validation studies included also

Additional Process Qualification Evaluations: Mixing Performance Qualification, Process Simulation Testing, Sterilizing Filter Validation and Shipping Validation.

2.4.3.3. Product specification

Specifications

Specification for the finished product includes control of identity, purity and impurities, biological activity and other general tests.

Except for the tests for subvisible particles, extractable volume, and sterility, which are tested only for finished product, (and capsid protein identity as well as DNA and protein composition, and product and process related impurities tested only for active substance), the test items are identical for active substance and finished product. The applicant provided a justification for each proposed specification acceptance criteria including batch analysis results, statistical considerations, and consideration of non-clinical and clinical aspects where applicable.

Overall, the proposed specification is considered adequate to ensure finished product quality taking into account the commitments of the applicant (**Recommendations 13, 14, 15**).

Analytical procedures

See active substance. Analytical methods specific to the finished product are compendial.

Reference standards

Four reference standards have been used throughout the development of etranacogene dezaparvovec finished product. The results of the qualification and characterisation of all four reference standards are appropriately provided. The protocol / acceptance criteria for the qualification of future reference materials have not been provided. Therefore, a variation procedure is foreseen before a new reference standard can be taken into use.

Batch analysis

Batch analyses data is appropriately provided in the dossier for historical and commercial batches.

Characterisation of impurities

The applicant conducted a risk assessment for elemental impurities in accordance with ICH Q3D guideline showing that there are no concerns. It is concluded that the risk is low and it is not necessary to include any elemental impurity controls in the finished product specification. This is acceptable.

A risk assessment regarding the potential presence of N-nitrosamines impurities in the active substance and finished product was provided during the procedure, as requested (Major Objection). This assessment concludes the risk is low and as a consequence there is no demonstrated need for testing either active substance or finished product for the presence of N-nitrosamines. This conclusion is endorsed.

2.4.3.4. Stability of the product

The applicant proposed a 24-month shelf-life based on 24 months of stability data at long-term storage conditions. The applicant has performed stability studies in accordance with the ICH Q5C guideline.

Stability studies were performed on batches representative of the commercial finished product with respect to manufacturing process and container closure system. Some of these batches were used in

clinical studies. The finished product batches were stored at long-term storage and at accelerated storage conditions.

Stability studies have been performed at the long-term storage condition (5°C ± 3°C) and at the accelerated condition. Forced degradation studies and photostability studies have also been performed.

The container closure system and the filled volume used for stability samples are identical to the final finished product.

To evaluate the stability-indicating capability of the analytical methods, a forced degradation study was performed. The results support the stability-indicating capability of selected analytical assays.

A photostability study was performed. The results indicated that the finished product is light-sensitive in primary packaging and that the proposed secondary packaging is effective in protecting the finished product from photodegradation.

Considering the totality of the data, the acceptable shelf life for Hemgenix is 24 months (2°C-8°C) protected from light.

In-use stability studies are discussed in the dossier and confirm that once diluted with sodium chloride 9 mg/mL (0.9%) solution for injection, Hemgenix can be stored at 15°C-25°C in the infusion bag protected from light. However, the administration of etranacogene dezaparvovec dose to the patient should be completed within 24 hours after the dose preparation. The stability after dilution was established for polyethylene/polypropylene (PE/PP) copolymer, polyvinyl chloride (PVC)-free infusion bags with sodium chloride 9 mg/mL (0.9%) solution for injection.

2.4.3.5. Post-approval change management protocol(s)

A post-approval change management protocol (PACMP) was submitted to introduce a process change in the active substance manufacturing process. The general approach proposed by the applicant was considered acceptable in general, additional recommendations were provided to the applicant during the procedure. However, the applicant decided to withdraw the PACMP.

2.4.3.6. Adventitious agents

Non-Viral Adventitious Agents

Microbial safety of etranacogene dezaparvovec against non-viral adventitious agents including mycoplasma, mycobacteria, spiroplasma, bacteria and fungi is considered sufficiently assured through testing of raw materials, cell banks and virus seeds together.

With regards to TSE, four materials of biological origin have been identified by the applicant: Certificates of suitability (when relevant) or TSE statements have been provided, assuring the TSE safety of these four materials.

Foetal bovine serum (FBS) was used in the preparation of pre-Master Seed Viruses. Certificates of suitability from EDQM were provided.

FBS and recombinant insulin were used at some point during the development of the MCB by the supplier of the cells which was stopped after 1992/1993. The applicant provided sufficient evidence that the producer cells pose a negligible TSE risk. This conclusion can be endorsed.

Viral Adventitious Agents

The control strategy for viral adventitious agents is performed at several levels: selection of raw materials, testing of starting materials for viral adventitious agents, testing of process intermediates at appropriate stages, and the inclusion of steps in the manufacturing process with virus reduction capacity.

With respect to the four raw materials of biological origin used in manufacturing, no risk of virus contamination was concluded based on their origin and/or their manufacturing process.

Two materials of biological origin were also used in the generation of the cell banks and the viral seeds. The manufacturing process of those materials is expected to inactivate viruses, although no proof of this has been presented. FBS used in the generation of the starting seed viruses was shown to be free from relevant bovine viruses. The approach of a risk assessment and testing or relevant viruses identified demonstrated freedom from viral contamination. Equally, viral seeds were also tested for viral contamination.

Another control layer is the testing of crude harvest and active substance. Testing occurs with indicator cell lines.

A virus risk assessment was performed to assess the risk of exposure by patients to adventitious agents which confirms a negligible risk. In summary, viral adventitious agents are considered sufficiently controlled at several levels (raw materials, cell banks, seed viruses and manufacturing process) allowing assurance of viral safety for the final product.

Overall, adventitious agents safety is considered sufficiently assured.

2.4.3.7. GMO

Hemgenix contains genetically modified organisms (GMOs). See Non-clinical section Ecotoxicity / environmental risk assessment.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

The quality dossier submitted for etranacogene dezaparvovec (Hemgenix) is generally well organised and provides an adequate description of the active substance and finished product, the manufacturing procedure and the control strategy defined to ensure a consistent production of the active substance and finished product of acceptable quality.

All the issues raised during the procedure have been resolved, but several Recommendations are made, some of them based on commitments proposed by the applicant.

The applicant applied for an exemption from retesting upon importation into the EU. The provided justification was not considered sufficient (Major Objection). In their response, the applicant agreed to commit to a staggered approach to transfer the analytical test methods to EU GMP-certified testing laboratories within a defined timeframe as per presented plan for the transfer of methods for finished product release testing in the EU **(Recommendation 1)**.

Recommendations are introduced for the introduction of testing of PCVs for future MCB and MSV and to provide remaining stability data for a WCB lot after testing within a defined timeframe **(Recommendations 2 and 3)**.

A major objection was initially raised regarding the adequacy of the process control strategy to guarantee consistency of the product. The applicant responded to this objection with additional details

on the process control strategy and several commitments to track process performance to confirm the process control strategy adequately assures consistent product quality. The applicant was requested to include in the CPV programme an analysis of the upstream infection step, and of the performance of two purification steps (**Recommendations 4, 5 and 6**). In addition, the applicant is recommended to estimate the range of MOI used for each batch based on the actual titre of infectious baculovirus. In view of the results obtained, the possibility of establishing an acceptance criterion MOI for better control of the infectious steps should be explored (**Recommendation 7**). The applicant also committed to perform formal hold time validation studies to collect additional data for extended hold times. Submission of the hold time validation study results will occur in terms of a post-approval variation procedure for extension of intermediate hold times. This applicant's commitment is included in the list of recommendations **(Recommendation 8)**.

Regarding the characterisation and control of the active substance, the applicant committed to complete the experiments to purify and functionally characterise and to incorporate a release assay for the purity attribute, proposing to introduce the method as a release test as a post-approval variation **(Recommendations 9 and 10)**. In addition, the revised method validation report for a release assay for process related impurities **(Recommendation 11)** and additional results for active substance batches on stability are to be provided post-approval **(Recommendation 12)**.

Regarding control of the finished product, the applicant committed to further revise the acceptance limits for some specification parameters once data from new finished product commercial batches are available **(Recommendations 13 and 14)**. Additional commitments have been made to introduce a release method for the attribute purity and to update the leachable study results **(Recommendations 15 and 16)**.

The applicant also committed to provide the GMP certificates for testing sites once available **(Recommendation 17)** (no pre-approval GMP inspection was required and these sites are included in the EMA re-inspection programme and subject to regular GMP inspections post-approval).

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall quality of Hemgenix is considered acceptable when used in accordance with the conditions defined in the SmPC. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines.

In conclusion, based on the review of the data provided, the marketing authorisation application for Hemgenix is considered approvable from the quality point of view.

The CHMP endorses the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

2.4.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommends the following points for investigation:

Description
1. The applicant commits to transfer the analytical methods and release testing to EU testing laboratories using a staggered approach. All finished product release testing should be conducted in the EU GMP-certified testing laboratories within defined timeframe following

Description

completion of analytical method transfer as per presented plan and successful Type II variation procedure outcome.

Active substance

2. The applicant is recommended to include testing of porcine viruses in the testing panel for future MCB and MSV.

3. The applicant is recommended to submit the next time points of the stability data for WCB lot upon availability after testing.

4. The applicant is recommended, as committed as part of the ongoing CPV programme, to analyse data from all commercial batches at a regular frequency to ensure the process performance and the process control strategy are appropriate to ensure product quality. Special attention should be paid to data for biological activity.

5. The applicant is recommended to perform additional studies to improve performance of a downstream purification step in the active substance manufacture.

6. The applicant is recommended to perform additional studies to improve the performance of a downstream step in the active substance manufacture to reduce residual impurities.

7. The applicant is recommended to perform a post-hoc analysis of infectious titre ranges observed in the upstream step. The possibility of establishing an acceptance criterion for better control of this step should be explored.

8. The applicant is recommended, as committed, to perform a formal hold time validation study to collect additional data for extended hold times at full-scale at each relevant process intermediate. The applicant confirms that the hold time validation study results will be submitted in terms of a post-approval variation.

9. The applicant is recommended to complete, as committed, the experiments for an assay for the attribute purity.

10. The applicant is recommended to develop and incorporate, as committed, a release assay for the attribute purity, proposing to introduce the method as a release test as a post-approval variation. The introduction of this methodology as release testing is planned within a defined timeline.

11. The applicant is recommended, as committed, to provide the revised method validation report for assay to measure process related impurities.

12. The applicant is recommended to provide the additional results for active substance batches on stability post-approval.

Finished product

13. The applicant is recommended, as committed, to revise the upper limit for finished product potency specification once additional data from finished product commercial batches manufactured are available.

14. The applicant is recommended, as committed, to reassess the finished product specification for the attribute biological activity once the data for finished product commercial batches tested using the newly validated method are available.

Description
15. The applicant is recommended, as committed, to introduce a release method for the finished product, once the analytical method validation is accomplished and the release criteria is established.
16. The applicant is recommended, as committed, to provide the finished product leachable study for the timepoints until the study is completed.
17. The applicant is recommended to provide the GMP certificates for two testing sites once available.

2.5. Non-clinical aspects

2.5.1. Introduction

The non-clinical development programme was initiated with AMT-060, which has a similar vector backbone as etranacogene dezaparvovec (AMT-061), but lacks the 2-nucleotide change in the hFIX coding sequence (enhancing the FIX activity of Padua FIX variant). A comprehensive set of pharmacology, biodistribution, and toxicology studies has been performed in mice and non-human primates (NHPs, in cynomolgus or rhesus macaques) to assess FIX expression and activity, biodistribution pattern, shedding, and safety including analysis of genome integration and paternal germline transmission of vector DNA. The pivotal biodistribution and toxicology studies were GLP-compliant. The IV route of administration was used in all non-clinical studies to mimic the intended clinical route of administration. The etranacogene dezaparvovec batches used in the non-clinical safety testing were representative of the final product used in clinical phase 2b and 3 studies. AMT-060 and etranacogene dezaparvovec were similar in terms of transduction efficacy, hFIX transcription and translation efficacy, biodistribution pattern and safety. Up to 4 to 6-fold higher FIX clotting activity was noted with etranacogene dezaparvovec administration in comparison to equal doses of AMT-060 in mice and monkeys.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

In support of the MAA of etranacogene dezaparvovec a range of non-clinical *in vivo* pharmacodynamic studies was conducted in wild-type mice, haemophilia B mice (B6.129P2-F9^{tm1Dws}; mice deficient for murine factor IX protein/activity) and cynomolgus monkeys. These studies were conducted with AMT-060, the initially developed product, as well as etranacogene dezaparvovec (AMT-061), a vector that bears the Padua-variant of FIX exhibiting higher clotting activity per unit FIX protein. PD endpoints were largely incorporated in biodistribution and toxicity studies and hFIX expression was followed up for up to 18 months after administration.

A proof of concept study (NR-060-11-007) was conducted employing AMT-060 and AAV5(92)-LP1-hFIXco, a predecessor thereof as a positive control, dosed from 1.0 x 10^{12} to 5 x 10^{12} gc/kg (AAV5(92)-LP1-hFIXco 2.5 x 10^{12} gc/kg only) to 15 weeks old male C57Bl/6 mice. After a 4-week observation period vector DNA and hFIX mRNA were detected at dose-dependent amounts in the livers of the mice. In addition, and also in a dose dependent fashion, hFIX protein was detected in murine plasma.

For the purpose of evaluating the efficacy of AMT-060 in a disease model, FIX knockout, B6.129P2-F9^{tm1Dws} mice that express minimal levels of endogenous FIX protein were administered 5x10^{11}, 5x10^{13} or 2.3x10^{14} gc/kg AMT-060 (NR-060-13-007). Endogenous FIX protein levels ranged from 6-18% of normal. Four weeks after administration concentrations of hFIX protein were detectable in a dose-dependent manner. In line with hFIX protein also FIX clotting activity increased with increasing doses (11-33%, 644-2280% and 1760-4780% of normal human plasma). Good correlation of hFIX protein levels and clotting activity was observed.

In order to follow transgene expression for a time period of up to 18 months, normal C57Bl/6 mice of different ages (neonates – 2 days old, weanlings – 3 weeks old, young adults – 6 weeks old and adults – 11 weeks or 6 months old) were dosed 2.3x10^{14} gc/kg AMT-060 and sacrificed at 18 months of age (NR-060-14-008). Human FIX protein was detectable in all age groups already 4 weeks after vector administration and throughout the entire study period. Neonatal mice had continuously low hFIX protein levels of less than 10% of normal and even less from 6 months after administration (around 4%). Weanlings had relatively stable hFIX protein levels of 748% of normal after 4 weeks with a moderate decrease over time to 269%. Adult mice with 6 weeks of age and older had in common a steep increase of hFIX protein levels of between 3316 and 5294% of normal which subsequently declined but remained stable at a level of around 1000% of normal until the end of the study period.

In order to investigate the effect of co-medication with prednisone, which is a potential scenario for the clinical setting, a single-dose toxicity and biodistribution study was conducted in normal C57Bl/6 mice that also included PD parameters, i.e. measurement of hFIX protein levels (NR-060-14-002). Mice received single doses of 5.0x10^{11}, 5.0x10^{13}, 2.3x10^{14} gc/kg or 2.3x10^{14} gc/kg plus 1 mg/kg prednisone three times per week. Human FIX protein levels were measured 8, 28, 90 and 180 days after administration and followed a dose-dependent pattern. Of note, hFIX was reduced to the level of the 5.0x10^{13} dosing group in the group that received 2.3x10^{14} gc/kg plus 1 mg/kg prednisone, whereas vector DNA levels in the livers of both 2.3x10^{14} gc/kg groups were comparable.

In the scope of a combined toxicity and biodistribution study in wild-type mice three doses of AMT-060 and AMT-061 (5x10^{11}, 5x10^{12}, 5x10^{13} gc/kg, all formulated with 0.02% PS-20) were additionally compared with respect to hFIX protein levels, as well as chromogenic and clotting activity (NR-061-18-002). In addition, in the high dose group AMT-060 and AMT-061 formulated without PS20 were included. Overall, no difference in hFIX protein levels was detected within one dosing level irrespective of the formulation. Only in the 5x10^{11} gc/kg dosing group AMT-061 appeared to be lower than in the AMT-060 group, however, standard deviations were very high in that group. With regard to FIX chromogenic and clotting activity, AMT-061 exhibited a 3- to 4-fold and 5- to 6-fold activity, respectively. No difference was determined between different formulations at the same dose level. Thus, it can be concluded that based on equal hFIX levels the activity of AMT-061 is clearly higher as compared to AMT-060.

A comparative *in vivo* analysis was performed with five AMT-061 batches that differed with regards to their *in vitro* potencies covering a range from 0.4-1.9 RU (NC-RPT-00006). Male wild-type mice were administered doses of 5x10^{11}, 5x10^{12} and 5x10^{13} gc/kg of each batch. Levels of hFIX protein as well as FIX activity appeared to be dose dependent and did not point towards differences in *in vivo* potency. Similarly, vector DNA levels in the livers varied dose-dependently and no consistent differences between batches were determined. Thus, differences in potency observed *in vitro* did not translate to *in vivo* potency or were blurred by differences in actual drug product concentrations as compared to nominal ones and varying transfection efficiencies within the 15 animals of each dosing group. Nevertheless, it can be concluded that despite up to almost 5-fold differences in *in vitro* potency, all batches produced a clear dose-related PD effect comparable within each dosing level.

Administration of a single dose of $5x10^{12}$ gc/kg AMT-060 to adult male cynomolgus monkeys resulted in hFIX protein expression for at least 13 weeks with an initial peak of up to 18% of normal human levels 1 week after administration and stabilisation of the protein level at about 5% of normal human levels (NR-060-12-003). Thirteen weeks after dosing the average vector DNA level was 1.3×10^{6} gc/µg DNA and average hFIX mRNA was 5×10^{4} copies/µg RNA.

In the scope of a biodistribution and toxicity study in cynomolgus monkeys the relationship between AMT-060 dose and circulating protein was analysed (Study NR-060-14-010). Monkeys (n=3 per dosing group) received doses of $5x10^{11}$, $5x10^{12}$, $2.5x10^{13}$ or $9.3x10^{13}$ gc/kg. Blood was collected regularly through week 26 and hFIX levels were determined by ELISA. Human FIX protein levels followed a similar pattern in all dose levels, i.e. an initial peak around day 8 after infusion and a more or less pronounced decrease (highest decrease in the middle dose levels) thereafter with stabilisation of the levels from about 8 weeks after administration up to the end of the study period at week 26. A clear dose dependence was observed with respect to hFIX levels ranging from 0.3% of normal human levels in the lowest to 15% in the highest dosing group. Whereas hFIX levels were relatively similar in all animals of the same dosing group for mid and low doses, two animals in the high dose group experienced a significant drop in levels from 31.3% and 23.9% to 4.9% and 0.5%, respectively. In correlation with the decline in hFIX levels, the respective animals were demonstrated to have developed anti-hFIX antibodies. Of note, only animals without pre-existing anti-AAV5 antibodies were included in the study. However, post-hoc analysis of the NHP sera with a more sensitive method revealed that all monkeys had neutralizing anti-AAV5 antibodies, which appeared not to have an effect on transduction or at least did not hinder efficient transduction. This was also demonstrated by correlating anti-AAV5 antibody titres with vector DNA levels in the livers and plasma hFIX levels.

In order to compare the PD properties of AMT-060 and AMT-061, doses of $5x10^{12}$ (AMT-060) and $5x10^{11}$, $5x10^{12}$ $2.5x10^{13}$, $9x10^{13}$ (AMT-061) gc/kg, respectively, were administered to cynomolgus monkeys (NR-061-17-001). All dose levels except for the highest and second highest AMT-061 dose produced an initial peak of hFIX protein with subsequent decrease and stabilisation until the end of the study period after 26 weeks or 13 weeks for the $5x10^{11}$ gc/kg group. In animals of the highest dose group protein levels increased until week 8 after administration followed by only little decrease thereafter. In the $2.5x10^{13}$ gc/kg group a second peak was observed at week 8 catching up with the highest dosing group with only one drop at the 24 week observation point. Both $5x10^{12}$ dosing groups performed equally in terms of hFIX levels. Human FIX activity was assessed by an one-stage aPTT and a chromogenic assay. As monkey FIX activity cannot be distinguished from that of hFIX, the baseline activity in monkeys was determined before administration of AMT-060 or AMT-061 and found to be around 50% of normal human levels. Overall, FIX activity analysed with either of the two assays was dose-related and correlated in general well with hFIX protein levels for the AMT-061 groups and reached up to 400% of normal human levels in the highest dose group. The dose of $5x10^{12}$ AMT-060 gc/kg resulted in no to very low FIX activity on top of baseline levels. In this group, a drop in FIX activity was observed after week 8 after administration. This drop in activity correlated with the incidence of anti-hFIX antibodies in one out of three animals of this dosing group. Comparison on an individual basis also revealed an inversely related correlation between hFIX-specific antibodies and hFIX protein levels. Of note, as the incidence of anti-hFIX antibodies occurred preferably in the $2x10^{13}$ gc/kg group (two out of 3 animals), no correlation to the dose administered appears to exist.

Two commercial assays, a chromogenic assay and a FIX-specific aPTT test, were set up for their use with mouse or monkey plasma. The criteria for assay validation are considered fulfilled for both assays and the assays are, thus, regarded suitable for the measurement of FIX activity in the preclinical species employed.

2.5.2.2. Secondary pharmacodynamic studies

No studies on secondary pharmacodynamics have been conducted which is acceptable considering the nature of the product and its mode of action.

2.5.2.3. Safety pharmacology programme

Safety pharmacology endpoints were included in the toxicity studies in cynomolgus monkeys with AMT-060 and AMT-061. With regard to ECG readings no effects related to the test article were observed with AMT-060.

2.5.2.4. Pharmacodynamic drug interactions

In order to assess potential effects of prednisone treatment that is applied in patients that develop transaminitis secondary to transfection of hepatocytes, mice that were dosed with 2.3×10^{14} gc/kg were co-administered with 1 mg/kg prednisone 3x per week (NR-060-14-002). While vector DNA levels of prednisone-treated mice were comparable to those in mice that did not receive co-medication, hFIX protein levels were reduced upon prednisone treatment.

2.5.3. Pharmacokinetics

Pharmacokinetics assessment of etranacogene dezaparvovec and AMT-060 were incorporated in pharmacodynamics and toxicological studies conducted in mice and non-human primates. An additional study on paternal germline transmission was conducted in male mice treated with AMT-060. This latter study is discussed in detail in the toxicology section of this report.

Pharmacokinetic studies focused on biodistribution, shedding and persistence of etranacogene dezaparvovec and AMT-060 in mice and NHPs.

Methods of analyses included assessment of vector DNA and vector-derived mRNA with qPCR and RT-qPCR, hFIX protein, anti-hFIX antibodies, and activity in mouse and monkey matrices. All assays were fit to purpose. Activity was assessed by two methods; activated partial thromboplastin time (aPPT) and chromogenic assays both measuring the total FIX activity (including vector-derived and endogenous activity). Anti-capsid AAV5 antibodies were assessed at pre-dose (screening) and at 25 weeks post treatment in monkeys.

The assays were validated according to recent guidance and in compliance to GLP when necessary, bridging validation was conducted to confirm comparability for the detection of AMT-060 and etranacogene dezaparvovec using the same method.

Dedicated studies on absorption were not conducted. This is acceptable with regard to this type of gene therapy medicinal product.

Biodistribution of AMT-060/etranacogene dezaparvovec was investigated as part of various PD and toxicological studies conducted in mice and NHP. The selection of tissues for biodistribution analysis differed between studies, but the following list was collected in all pivotal, GLP-compliant studies in mice and cynomolgus macaques: liver, adrenal glands, brain, heart, kidneys, lungs, lymph nodes, muscle, pancreas, salivary glands, spleen, thymus, thyroid, testes, epididymis, seminal vesicles, and prostate. To inform on clearance/shedding of the vector DNA, plasma or serum, urine, saliva, and semen samples were collected from cynomolgus macaques treated with either AMT-060 or etranacogene dezaparvovec. Vector DNA in plasma was also evaluated in mice.

In the non-GLP compliant study NR-060-11-007, distribution of AMT-060 to the liver was assessed in C57Bl/6 mice. Mice were dosed IV up to 5.0×10^{12} gc/kg body weight. This resulted in dose-dependent transduction of the liver, with liver vector DNA mean measured to be $8.0x10^4\pm6.9x10^4$ at the highest dose tested.

In the non-GLP study NR-060-14-008, newborn, juvenile and adult male C57Bl/6 mice were dosed IV with a dose of 2.3×10^{14} gc/kg of AMT-060 at an age of either 2 days (neonatal), 3 weeks (weanling), 6 weeks (juveniles), 11 weeks (adult), or 6 months (aged) old to study long-term persistence of liver vector DNA. Data revealed long-term persistence of vector DNA in the liver of mice treated at the various ages listed above. However, animals treated at 6 weeks and older showed higher DNA levels four weeks after administration than neonatal mice and weanlings (1-2 log lower DNA levels). After 18 months DNA vector levels were approximately one log lower than the levels measured four weeks after treatment, with exception of animals dosed at day 2, which showed a 2-log reduction. However, vector DNA was still detectable in all age groups' liver samples.

In the GLP-compliant studies NR-060-14-002 and NR-060-13-006, biodistribution of AMT-060 to liver and off-target tissues was determined after a single IV injection to male mice with a 26-week follow-up period. AMT-060 was dosed up to 2.3×10^{14} gc/kg, with a second high dose group receiving prednisone as co-treatment (3x/week, 1 mg/kg) to see if co-administration could alter biodistribution.

Tissue and blood samples were collected on day 8 and day 180 after dosing. Vector DNA was detected in all tissue samples tested in the high dose group. The highest levels of vector DNA were measured in the liver on both time points tested, followed by lung and spleen (levels within one log compared to liver on day 8, but >1 log on day 180). DNA levels on day 180 decreased in all tissues when compared to levels found on day 8. Prednisone treatment did not show a significant impact on biodistribution or DNA levels in this study.

In the GLP study NR-061-18-002, male mice received a single IV dose of either etranacogene dezaparvovec or AMT-060 in different formulations (with and without PS-20), followed by a 13-week follow-up period, to support direct comparison between the two products and differences in formulations. Biodistribution of the two vectors to liver and off-target tissues was determined at three dose levels ($5x10^{11}$, $5x10^{12}$, $5x10^{13}$ gc/kg).

Vector DNA in plasma decreased from 10^{10} copies/ml on day 1 post-dose to 10^4 copies/ml after 13 weeks and levels were comparable between the two products and formulations. Distribution of vector DNA to the liver increased dose-dependently and no differences were observed between the same dose levels of the two products.

Vector DNA was further detectable in all off-target tissues tested with highest concentrations found in adrenal glands, heart, kidney, and spleen. With the exception of the adrenal glands, vector DNA levels in off-target tissues were ~100-fold lower than in the left liver lobes. Again, no significant differences were observed between AMT-060 and etranacogene dezaparvovec. The addition of PS-20 to the formulation did not impact on biodistribution.

In the non-GLP studies NR-060-12-003 and NR-060-11-009, biodistribution to the five liver lobes of rhesus macaques was investigated after single IV administration of AMT-060 at a dose of 5×10^{12} gc/kg with a 90-day recovery period. Homogenous distribution of vector DNA was observed among the liver lobes in all treated animals.

In the GLP-compliant study NR-060-14-010, biodistribution of AMT-060 was determined after single IV dosing of male cynomolgus monkeys dosed up to $9.3x10^{13}$ gc/kg, followed by a 26-week investigation period. Additionally, vector DNA delivery and transgene expression in liver (non-GLP; NR-060-14-006) and off-target tissues (non-GLP; NR-060-14-011) were investigated in these animals.

Blood was collected to determine vector DNA levels in serum. Secretion and excretion of the vector DNA was measured in saliva and urine. Fluid samples were taken at pre-dose, at week 1, 2, 4, 8, 12, and 26. Semen samples were collected on day 180. At necropsy 19 different tissues were collected for vector DNA analysis by validated qPCR. Four liver lobes were analysed. The hFIX mRNA analysis by RT-qPCR was conducted on a selection of tissues based on vector DNA levels returned by qPCR.

Vector DNA levels in serum, saliva and urine decreased over time, with serum showing the highest concentrations at all time points measured. Vector DNA levels were below LOD after week 8 in saliva and urine. Low levels of vector DNA were detectable in the highest dose group's semen samples, whereas in the lower dose group levels were close to the LOD.

All animals showed dose-dependent, homogenous and comparable distribution of vector DNA among their liver lobes. Dose-dependency was also observed in all other tissues investigated. In the highest dose group (9.3×10^{13} gc/kg) similar levels were detected in the liver and adrenals, followed by the spleen, 26 weeks after dosing. In all other tissues, DNA levels were >1 log lower than in the liver.

However, in accordance with the liver-specific promoter, hFIX mRNA levels were highest in the liver and correlated with the vector dose. All other tissues examined (including adrenal glands and spleen) showed mRNA expression below the LLOQ.

In the GLP-compliant study NR-061-17-001, biodistribution of etranacogene dezaparvovec, directly compared to AMT-060, was determined after single IV dosing of male cynomolgus monkeys dosed up to $9.3x10^{13}$ gc/kg, followed by a 13 to 26-week observation period.

Vector DNA levels in serum decreased at a bi-phasic rate. The plasma half-life (t½) at the dose (2.5×10^{13} gc/kg) closest to the recommended human dose was determined to be around 25 days, with comparable plasma curves found between etranacogene dezaparvovec and AMT-060. Low levels of vector DNA were detected after 6 months in all dose groups except the lowest one, while DNA shedding to urine was only detectable until 3 months post dose.

Biodistribution occurred to all tissues tested, with liver again showing the highest levels as already observed in previous studies. Highest off-target levels (>1 log lower than in liver) were detected in adrenal glands and spinal cord (with the latter not tested in previous study). Liver concentrations measured for etranacogene dezaparvovec showed dose-dependency and etranacogene dezaparvovec and AMT-060 showed similar levels for the compared dose groups in all organs and tissues.

hFIX mRNA levels in the liver showed dose-dependency and were comparable for etranacogene dezaparvovec and AMT-060. mRNA at low levels was also detectable in adrenal glands, although estimated to be 30-65-fold lower than in liver at similar vector DNA levels. At the highest dose administered low levels of mRNA around the LLOQ were moreover detectable in the spleen, kidney, spinal cord and heart samples of some animals. Altogether, these data support the liver-specific promoter of the construct. Low levels of off-target expression have also been demonstrated for other AAV-mediated gene therapies with transgene expression regulated by tissue-specific promotors (Prasad 2011). However, expression around LLOQ in other tissues is not expected to be associated with adverse effects.

Transduction of liver cells was estimated to be between 17-46 percent, depending on the dose administered, using FISH. Again, no significant differences between etranacogene dezaparvovec and AMT-060 were observed.

Overall, data on the biodistribution of etranacogene dezaparvovec and AMT-060 did not reveal unexpected/adverse findings on tissue distribution of the vector DNA or mRNA expression thereof. The vector DNA biodistribution, transduction and hFIX mRNA expression profiles were comparable between AMT-060 and etranacogene dezaparvovec at equal dose of 5×10^{12} gc/kg. The AUCs were 3.7×10^{10},

and 3.76×10^{10} gc × hr/mL for AMT-060 and etranacogene dezaparvovec, respectively. While most off-target tissues showed detectable levels of the vector DNA, liver presented the highest levels in all studies conducted, with mRNA measurements confirming the liver-specificity of the promoter used. Both, vector DNA levels as well as mRNA levels, were observed to increase with dose and to decrease over time, however still detectable 26-weeks after administration in liver of tested animals. Dose-dependency and decrease of vector DNA levels was also observed for all off-target tissues investigated, with no tissue or organ indicating accumulation over time. Differences in the formulation (PS-20) and co-treatment with prednisone did not significantly alter biodistribution of the vector.

Triggering of antibodies against the capsid and hFIX was evident in the monkey studies. Albeit the inter-animal variability (small scale monkey studies), triggering anti-hFIX antibodies had tendency to be affected by the vector dose. The applicant confirmed that there was no difference in the antibody formation against FIX with AMT-060 compared to etranacogene dezaparvovec with the Padua hFIX variant in mice or in NHPs. AMT-060 and etranacogene dezaparvovec were comparable in their presence in the liver, biodistribution and hFIX protein levels in blood at equal doses. Thus far, no antibodies against hFIX have been noted in the clinical trials.

AMT-060 and etranacogene dezaparvovec were comparable in their presence in the liver, biodistribution and and hFIX protein levels in blood at equal doses. Thus far, no antibodies against hFIX have been noted in the clinical trials.

2.5.4. Toxicology

The toxicology data package of etranacogene dezaparvovec and AMT-060 included single IV dose toxicity studies up to duration of 6 months with 6 months observation period in C57Bl/6 mice and cynomolgus macaques, and a reproduction and developmental toxicity study in mice. IV-route of administration, the clinical route, is used in all toxicological studies.

Doses ranged from a dose supporting a low pharmacological effect level to a dose corresponding to approximately 10- and 5-fold the recommended human dose of 2×10^{13} gc/kg, as tested in mice and cynomolgus macaques, respectively.

Clinical chemistry, haematology/clotting, and cytokine analysis were conducted. To evaluate the haemostatic safety related to high levels of circulating hFIX and FIX activity, thrombin/antithrombin III (TAT) complex, and D-dimer levels were measured in the toxicity studies. AMT-060 and etranacogene dezaparvovec showed a comparable safety profile when tested side-by-side in mice and NHPs.

2.5.4.1. Single dose toxicity

The single IV dose toxicology studies and the analysis of the safety parameters were performed under GLP compliance.

Etranacogene dezaparvovec and AMT-060 were well tolerated in mice and monkeys. The studies did not reveal any adverse target organ toxicities associated with the administration and biodistribution of the vector, or as a consequence of high-level expression and activity of the hFIX-Padua protein. The overall no-observed-adverse-effect-level **(**NOAEL) for etranacogene dezaparvovec in the NHPs is 9×10^{13} gc/kg, the highest dose tested. 9×10^{13} gc/kg corresponds to the 385% of normal human plasma FIX activity level, being approximately 10-fold above the average FIX activity level achieved with etranacogene dezaparvovec at the recommended human dose of 2×10^{13} gc/kg (mean ± SD 36.90 ± 21.40% of normal FIX activity levels).

In mice, minimal pulmonary thrombi were observed with 5×10^{13} gc/kg dose (one animal treated with etranacogene dezaparvovec and one with AMT-060), and could be pharmacology-related, a consequence of the high FIX activity levels (approximately 1600% of normal for etranacogene dezaparvovec and 300% of normal for AMT-060). The differences in FIX activities between etranacogene dezaparvovec and AMT-060 did not reflect on thrombosis incidences in mice studies.

The most notable findings in NHPs were a transient mild elevation of liver enzymes AST (up to 4.5× mean control value) and ALT (up to 3.0× mean control value) observed on day 2 - 4 in all dose groups which is likely related to the viral vector load in the liver. Plasma liver enzyme activities were normal from Day 8 onwards. Effects on the clotting cascade were noted with 9×10^{13} gc/kg in NHPs. APTT was slightly shortened, while PT was marginally longer, likely a consequence of the high FIX clotting activity levels (endogenous macaque and vector-derived combined) up to ~500% of normal human plasma FIX levels. Plasma TAT complex and D-dimer levels were not elevated and there were no associated histopathological findings. Overall a similar toxicity profile for AMT-060 and etranacogene dezaparvovec, suggests no overstimulation of coagulation with hFIX Padua variant in comparison to wt hFIX.

No notable histopathological findings were recorded. After administration, the vector distributed widely and vector DNA was found also in the central nervous system including brains and spinal cord. The dorsal root ganglia toxicity (albeit currently with unknown clinical relevance) has been recently reported after administration of high dose of AAV vectors in the NHPs. The applicant confirmed that dorsal root ganglia toxicity was not included in histopathological analyses. No adverse findings in spinal cord (cervical, lumbar, and thoracic) histopathology, including absence of spinal cord axonopathy, were reported in the NHP study with etranacogene dezaparvovec dose up to 9×10^{13} gc/kg, with a 26-week follow-up. The dorsal root ganglia analysis was not included in the histopathology, but is to be investigated in juvenile NHP studies as part of the future PIP. These results will substantiate the current knowledge.

The addition of PS-20 to the test item formulations of AMT-060 or etranacogene dezaparvovec had no influence on the safety profile.

IgG antibodies against the AAV5 capsid proteins were detected after dosing even at the end of the 6-month observation period. Antibodies against hFIX was also detected in NHPs.

2.5.4.2. Repeat dose toxicity

No repeated dose toxicity studies were conducted. This is acceptable, as only a single etranacogene dezaparvovec administration is intended in patients, obviating the need for repeated dose toxicity studies with such kind of gene therapy. Furthermore, the high immunity against the AAV5 vector obtained after the first administration would complicate subsequent administrations, or even make them impossible.

2.5.4.3. Genotoxicity

Vector integration after single IV administration of AMT-060 was observed following evaluation of liver samples from mice (20 samples, dosed up to $2.3\text{x}10^{14}$ gc/kg with and without prednisone) and cynomolgus macaques (12 samples, dosed up to $1\text{x}10^{14}$ gc/kg), with tissue collected at sacrifice 26 weeks post dose.

Integration site analysis was performed using linear amplification-mediated PCR (LAM-PCR) and non-restrictive (nr) LAM-PCR followed by deep-sequencing. Liver samples were obtained from the right lobe of the non-human primates or whole liver of mice. Vehicle control groups were included in both studies.

In mice (n = 4) injected with rAAV5-hFIX at a dose of 2.3 x 10^{14} gc/kg body weight, corresponding to approximately 10-fold higher dose than the clinical dose in human, 3 x 10^{7} gc/µg host DNA were determined at the end of a 26 week observation period, corresponding to a vector copy number/liver cell (VCN) ratio of approximately 180. In the murine DNA samples, 266 unique IS could be identified. This indicated that out of 1,000 liver cells approximately 1.6 may carry an integrated vector. On a per vector genome basis, less than 1 vector out of 100.000 was assumed to be genome-integrated in mice at this dose. In NHP (n = 3) injected with 2.5 x 10^{13} gc/kg body weight, a dose approximately similar to the human dose, 1.67 x 10^{6} gc/µg host liver DNA were detected, along with an average of 35 IS. This corresponded to a VCN of approximately 11, along with 2.1 integrations for every 100.000 vector copies at 26 weeks post-dose. Of note, in an available human liver biopsy collected one year post–dose from a study participant (see also the clinical safety section), 6.25 x 10^{5}gc/µg host liver DNA and 26 IS were determined, indicating that for every 100.000 vector genomes 4.2 may be genome-integrated, thus similar numbers as observed in animal studies.

The AAV-vectors are predominantly in episomal concatemeric forms. Low level of integration of AAVs are known to occur and this is an identified risk. The integration analysis conducted with liver tissue obtained from NHPs and mice 6 months after administration with AMT-060 confirmed a measurable but low level of genome integration in both species' liver. The retrieved integrants were randomly distributed throughout the host genome in the NHPs, while in mice some clustering of AAV integration sites was found in liver. The observed integration profile was not associated with genes implicated in clonal outgrowth or malignant transformation. Of note, histopathological evaluation of the liver tissues from mice and NHPs did not reveal any abnormalities that could otherwise point to potential carcinogenicity, such as hypertrophy or hyperplasia at 6 months post AAV-administration.

2.5.4.4. Carcinogenicity

Dedicated studies on carcinogenicity were not conducted with AMT-061 (nor AMT-060).

2.5.4.5. Reproductive and developmental toxicity

As males comprise the majority of the patient population to be treated, the applicant conducted a paternal germline transmission study in mice to address gross adverse effects on the (male) reproductive performance. Treated males were paired with untreated females on day 6 after male dosing. Although high levels of AMT-060 were detected in all tissues examined (epididymis, seminal vesicle, sperm, and testes) in male mice, no AMT-060 was detectable in untreated females nor the offspring (uterus, foetuses and placenta were examined). Thus, no paternal germline transmission was detected in this study. No adverse effects on reproductive organs were detected in general toxicology studies.

As haemophilia B is almost exclusively limited to male patients, and as the current MAA only comprises adult patients, dedicated EFD and PPND studies are not required. Assessment of juvenile toxicity is not warranted in the presented setting.

2.5.4.6. Toxicokinetic data

Refer to PK section.

2.5.4.7. Local tolerance

No dedicated local tolerance studies were conducted.

No notable findings were observed at microscopical examination of the injection site which was part of the general toxicity studies conducted.

2.5.4.8. Other toxicity studies

Antigenicity endpoints were included in single dose toxicity studies, capsid antigens generally caused high titres of anti AAV5 antibodies. In addition, anti-human FIX antibodies were observed inconsistently in NHP.

2.5.5. Ecotoxicity/environmental risk assessment

Hemgenix (etranacogene dezaparvovec) is an AAV-based vector with an expression cassette containing hFIXco-Padua under the control of a liver-specific promoter (LP1). Etranacogene dezaparvovec belongs to AAV serotype 5 (AAV5) determined by the capsid. The genetic modifications introduced during the development of etranacogene dezaparvovec have not affected natural AAV5 host range and tissue tropism.

The modified organism (AAV) is non-replicative and the transgene and its regulatory sequences are non-toxic or harmful to humans.

Based on the current available data, infectious particles are only demonstrated in serum during the first days after administration (Favre et al, 2001). Consequently, shedding of infectious GMO particles is highly unlikely, but cannot be excluded. In the hypothetical case that shedding of infectious GMO does occur, the risk to the environment is considered negligible. Consequently, standard hospital hygiene measures are sufficient, and no additional measures are deemed necessary after etranacogene dezaparvovec administration. Accordingly, no discharge criteria have been identified that relate to potential environmental risks. Patients may leave the hospital as soon as the post-administration monitoring has passed, unless a prolonged stay is medically justified by the healthcare professional.

To ensure that other people without haemophilia B are not exposed to Hemgenix DNA through a shedding process, the following risk minimisation measures are introduced (SmPC 4.4. Special warnings and precautions for use, and PIL / shedding): Patients treated with etranacogene dezaparvovec should not donate blood, or organs, tissues and cells for transplantation to minimise the risk of exposure to non-target individuals.

Considering the evaluation of the characteristics of Hemgenix with respect to their potential of causing adverse effects to people or the environment, the potential consequences that might result from the occurrence of these effects and the likelihood that these effects occur, can be concluded as negligible.

2.5.6. Discussion on the non-clinical aspects

A comprehensive panel of PD studies in wt-mice, FIX-deficient mice and cynomolgus monkeys was conducted in support of the MAA of etranacogene dezaparvovec. Overall, the results of these studies demonstrate that etranacogene dezaparvovec as well as the predecessor product AMT-060 are capable of efficiently transducing hepatocytes and, in turn, of increasing or restoring FIX protein levels as well as clotting activity in a dose-dependent manner in wt-animals and haemophilia B mice, respectively. Doses administered covered 5×10^{11} to $2.3x10^{14}$ gc/kg. A study employing wt mice ranging from neonates through weanlings and adults demonstrated that AMT-060 is capable of long term restoration

of stable FIX levels for at least 18 months after administration in normal mice. Notably, the treatment was hardly efficient in neonatal mice due to reasons that remain elusive. On the other hand, the vector efficacy was quite comparable in all other age groups, disregarding the initial peak observed in all adult age groups.

It was noted that generally only nominal (theoretical) dose values (gc/kg) were indicated, however, these can widely vary from real values, i.e the actual concentration of gc/mL. The applicant clarified that batches used for non-clinical studies were quantified by qPCR.

As stated by the applicant the employed chromogenic assay as well as the aPTT test do not discriminate between monkey and human FIX as well as murine FIX. Therefore, the results of all non-clinical studies reflect combined animal baseline FIX activity plus human FIX activity.

The applicant described that lower hFIX levels were detected in mice concomitantly treated with prednisone as compared to animals without steroid treatment. A similar observation was made in patients that were treated with steroids, however, only upon elevation of transaminase levels and not prophylactically. Due to various factors potentially influencing protein expression in patients, the decrease in FIX levels in combination with steroid administration cannot unequivocally be assigned to one of them. The mechanism responsible for the lower hFIX levels as a consequence of prednisone treatment remains elusive.

In the scope of a biodistribution and toxicity study in cynomolgus monkeys the effect of pre-existing anti-AAV5 antibodies on transduction efficacy was investigated. Interestingly, under the conditions of this study pre-existing neutralizing anti-AAV5 antibodies neither affected transduction efficiency nor hFIX levels, whereas emerging anti-hFIX antibodies clearly lowered plasma hFIX levels. The latter observation was limited to animals treated with $9.3x10^{13}$ gc/kg and, thus, appeared to be dose-dependent. Somewhat different results regarding anti-hFIX antibodies were observed in a different study in cynomolgus monkeys. Also in this study the highest incidence for anti-FIX antibodies was observed in the highest dose group, the inhibiting effect of these antibodies, however, was most pronounced in the lowest dose group. It is not considered valuable to follow up these observations on a non-clinical level as animal models are regarded to be of limited immunological predictivity for humans.

After IV delivery, etranacogene dezaparvovec and AMT-060 distributed widely to the liver and also to extra-hepatic tissues (including testis, lungs, heart, spleen, kidneys, bone marrow, lymph nodes, adrenal gland, brain and spinal cord). Highest vector concentrations were found in liver, and also highest hFIX mRNA was found in liver (transcription driven by the liver specific promoter). In the extra-hepatic tissues, the vector DNA copies detected were in general proportional to the dose administered.

AMT-060 instead of etranacogene dezaparvovec was used in several toxicological studies provided. This is regarded acceptable as AMT-060 differs only in two base pairs/one amino-acid to the final GTMP AMT-061 and thus, in the following toxicological section this deviation will only be further discussed, if specific concerns are raised based on this issue. Generally, these changes are not regarded to impact biodistribution nor the toxicological profile and studies conducted with AMT-060 are thus deemed representative. In some of the general toxicology studies conducted with AMT-061, an additional AMT-060 arm was included for comparison and to further strengthen the assumption that despite the introduced changes to etranacogene dezaparvovec, data obtained for AMT-060 still provide valid toxicity data.

Etranacogene dezaparvovec was well tolerated in mice and monkeys. The most notable findings were transient increases in the liver enzymes, and pharmacology activity-related effects on clotting parameters (shortened aPTT, longer PT), and occasional thrombi. NOAEL in the mice and NHPs was the

highest dose tested. NOAEL in NHPs was 9×10^{13} gc/kg, which resulted in 10-fold above the average FIX activity level achieved with etranacogene dezaparvovec at the recommended human dose of 2×10^{13} gc/kg, and corresponding to 385% of normal human plasma FIX activity level. Overall, a similar toxicity profile for AMT-060 and etranacogene dezaparvovec suggests no overstimulation of coagulation with the hFIX Padua variant compared to WT hFIX. Due to wide distribution after IV administration, vector DNA was found also in the central nervous system including brain and spinal cord. Dorsal root ganglia toxicity analysis was not included, but is going to be evaluated in juvenile animal studies as part of the PIP.

The addition of PS-20 to the test item formulations of AMT-060 or etranacogene dezaparvovec had no influence on the safety profile.

IgG antibodies against the AAV5 capsid proteins were detected after dosing even at the end of the 6-month observation period. Antibodies against hFIX were also detected in NHPs.

In the integration studies conducted using liver tissue of mice and NHP, a total of 13,949,235 sequences were analysed, resulting in 8646 unique IS for the mice and 1541 unique IS for NHP. While the number of IS showed to be dose-dependent in mice, an increase in the number of IS was only observed for high dose group in NHP. While IS were randomly distributed throughout the host genome in the cynomolgus, some intense clustering of AAV IS (= common integrations sites (CIS)) was observed in mice, with clustering of >30 IS (with the two most abundant CIS even >60 IS). Specific analysis of those CIS revealed the liver specific Alb (Albumin) gene, the Ttc39c gene (tetratricopeptide repeat domain 39c), the mouse specific Esp38 gene (exocrine gland secreted peptide 38), and the gene Lrrc4c (leucine-rich repeat-containing protein 4C), to show highest IS clustering. The most prominent CIS regions were located within the Albumin locus and the TTC39c gene, with a CIS order of 63. Both genes are known to be expressed in the liver. Integrations near the Albumin locus have also been reported in rAAV integration datasets from different animal models (Chandler et al 2015; Gil-Farina et al 2016). This observation is in accordance with previous studies that demonstrated a higher incidence of rAAV integrations within actively transcribed genes containing open chromatin. Genes showing hepatic activity are often also associated with hepatotoxicity. In the provided data sets CIS were accordingly detected in Alb and Cyp2e1 in mouse samples and CYP3A4, SERPINA1 and PDCD1 in NHP.

Literature states that CIS with IS <5 often occur by chance and thus are unlikely to have any biological relevance (Wu et al, 2006). In NHP only one CIS showed an order of >5 IS (i.e., order of 6 IS) and was located in the CYP3A4 gene. Overall, the data presented shows intense clustering of IS for few (above listed) genes only in mice, with predominance of lower order CIS in NHP, thus indicating that AMT-060 vector integrations generally to not tend to target specific genomic regions.

Analyses of respective genes with three different cancer gene databases (Cancer Gene Census (CGC), cBioPortal, Retroviral Tagged Cancer Gene Database (RTCGD)) did not list any of the concerned genes to have previously been associated with cancerogenesis. According to the applicant, no enrichment of integrations next to or within genes listed in cancer gene databases (CGC, cBioPortal, RTCGD) has been observed for either mouse or NHP. A significant increase of unique IS near genes listed in CGC and cBioPortal was however observed in mice.

Moreover, no preferred integration in the proto-oncogenes MECOM, LMO2 or HMGA2, previously implicated in insertional oncogenesis, was observed.

Obtained data did not reveal any signs pointing towards *in vivo* clonal selection, and the applicant thus concludes that altogether data do not indicate any specific carcinogenicity concerns. Although it can be agreed that the data presented does not hint towards clonal selection after AAV integration, no long-term data (>26 weeks) on this issue is currently available with AMT-060/061.

In conclusion, the data provided by the applicant show that integrations in the host genome do occur and have to be considered since the proposed human dose of vector administered will lead to a cumulative high number of integrations, at a single patient level as well as at a population level. Dose is considered a pivotal factor with regards to the frequency of integration and with that a main contributor to the risk of insertional oncogenesis. However, other factors affecting the integration profile and/or frequency may contribute to this risk as well, including the type and (diseased) state of the tissue, the design of the vector, and vector production itself.

Considering the AAV vector by itself, AAV5 vectors (including AMT-061), are replication defective and contain no viral genes. AAV vectors persist for prolonged periods as episomal structures and integrated copies of AAV vector genomes are currently assumed to be rare. However, there are observations of liver integration of AAV genomes in various animal models. Relevance to human risk is confounded by inter-study variability, vector construct dependencies, murine specific integration sites (e.g. Rian locus) and murine specific physiology (higher ploidy than typical liver diploid cells, contribution of disease progression, mouse strain specific spontaneous tumour formation) (Zhong, 2013; Chandler, 2015; Bell, 2006).

Extensive studies with AAV2 and AAV1 vectors in rodents (Schnepp, 2003; Inagaki, 2008; Li, 2011), rabbits (Schnepp, 2003), nonhuman primates (Nowrouzi, 2012) and in human subjects who were administered Glybera (an approved gene therapy product) (Kaeppel, 2013) lead to the estimation that the integration frequency of AAV vectors is several orders of magnitude lower than the spontaneous rate of mutation for human genomes (Cole, 1994) so that the likelihood of insertional mutagenesis by AAV vectors may be low.

However, in another publication authors found clonal integration of wild-type AAV2 in 11 of 193 hepatocellular carcinomas (HCCs). AAV2 integrations occurred in known cancer driver genes, namely CCNA2 (cyclin A2: four cases), TERT (telomerase reverse transcriptase: one case), CCNE1 (cyclin E1: three cases), TNFSF10 (tumour necrosis factor superfamily member 10: two cases) and KMT2B (lysine-specific methyl transferase 2B; one case), leading to overexpression of the target genes, and consequently, oncogenicity events. Authors concluded that wild-type AAV2 viruses are DNA viruses associated with oncogenic insertional mutagenesis potential in human HCC (Nault, 2015). The relevance of these finding to recombinant AAV5 viruses that lack the Rep genes required for integration, as employed in Hemgenix, is unknown.

Clonal populations of FVIII-producing cells harbouring vector DNA integrations were observed 10 years post dose in a haemophilia A dog study, in which animals were treated with AAV8/9 gene therapy. In this study clone selection was seen in 5/6 dogs, whereby 44% of the integrations were located near genes involved in cell growth (Nguyen, 2021). Even though no tumours were identified in this study, these findings could indicate pre-stages of malignancy.

Another example was reported in a study, which found that a pre-existing pathology (induced liver damage) had an impact on AAV vector-induced HCC (Dalwadi, 2021). The authors highlight that increased hepatocyte proliferation, coupled with inflammation, contributed to a higher incidence of HCC.

In a previous study, AAV-induced oncogenicity/tumourigenicity was related to site-specific integration of the vector into the Rian locus present in juvenile mice only (Chandler, 2017). However, more recent publications (Dalwadi, 2021; Ferla, 2021) link administration of AAV gene therapies also in adult mice to liver tumourigenicity (and thus also to potential insertional oncogenesis associated with other murine loci).

Although, so far, there is no direct link to resulting disease (in humans), it may be assumed that integration of AAV will at least affect liver biology. Considering the (unique) integration events

observed in the vicinity of cancer related genes in mice, the frequency of the events, and recent data obtained in different animal models (mice and dog) with similar vectors, it is important to follow-up patients treated with Hemgenix. Further, it is of even higher importance to adapt the follow-up measures for patients with a known risk for liver transformation. Even though the dimension of this risk in patients remains currently unclear, nonclinical studies of other AAV-based therapies demonstrate that AAV-transgene integration can potentially manifest in tumorigenesis. In human clinical trials, no cases of liver cancer linked to rAAV gene therapy have occurred.

Even though, this potentially severe and lifelong risk of insertional mutagenesis and subsequently carcinogenesis after intravenous Hemgenix administration has been accordingly depicted in section 4.4. and 5.3. of the SmPC and in the Risk Management Plan.

In the paternal germline transmission study, male mice were dosed with 2.3×10^{14} gc/kg AMT-060 six days before mating. Justification for the dosing regimen is assumed to be based on study NR-060-14-002 where C57BL/6 mice were dosed up to the same concentration and biodistribution was investigated in various tissues at day 8 and day 180 after dosing. At day 8, levels of AMT-060 vector DNA were detectable in all animals in all investigated tissues, including epididymides, seminal vesicles and testes. In the paternal transmission study itself, biodistribution of vector DNA to the above listed tissues plus sperm was detected in male animals on Day 20 after dosing, but no such data was provided for the time point of mating (D6). Justification for the selection of dose levels and the timing of dosage and pairing was provided by the applicant. AMT-060 was present in epididymides, seminal vesicles and testes on D6 in study NR-060-14-002, and in epididymides, seminal vesicles, testes and sperm on D20 after dosing in the presented study. The time point was chosen based on the expected presence of peak levels of qPCR-detectable vector DNA in testes and epididymis, including the maturing spermatozoa, which was subsequently confirmed in the 6-month toxicity study in mice with AMT-060 (at the Day 8 sacrifice). Reference to recent literature was also provided. It is acknowledged that vector DNA was neither detected in females nor fetuses in this study.

The risk of etranacogene dezaparvovec to third parties or to the environment is considered negligible.

2.5.7. Conclusion on the non-clinical aspects

Overall, the primary pharmacodynamic studies provided adequate evidence that murine and monkey hepatocytes are efficiently transduced by etranacogene dezaparvovec and produce hFIX dose-dependently, enabling sustained and durable hFIX activity levels.

From the pharmacokinetic point of view, non-human primates were the most relevant species for non-clinical efficacy and safety studies, as natural hosts for AAVs. The biodistribution of etranacogene dezaparvovec was determined by route of administration (IV) and capsid (AAV5).

Overall, the toxicology programme revealed that etranacogene dezaparvovec was well tolerated in mice and NHPs. AAV stays largely episomal, but a low level of integration was noted in both mice and NHPs.

The potentially severe and lifelong risk of insertional mutagenesis and subsequently carcinogenesis after intravenous Hemgenix administration has been included in the product information and Risk management Plan.

Etranacogene dezaparvovec can be granted a marketing authorisation from a non-clinical point of view.

The CHMP endorses the CAT assessment regarding the conclusions on the nonclinical aspects as described above.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Table 1. Overview of Clinical Studies

Study	Product / Dose	Study Status	Efficacy Variables
CT-AMT-060-01: A phase 1/2, open-label, uncontrolled, single-dose, dose-ascending, multicentre study investigating an AAV vector containing a codon-optimized human FIX gene (AAV5-hFIX) administered to adult subjects with severe or moderately severe hemophilia B Sites: 7, countries: 3 (DE, DK, NL) Subjects require 52 weeks of Post-treatment Follow-up, followed by 4 years of LTFU	AMT-060 Cohort 1: 5 subjects received 5×10^{12} gc/kg Cohort 2: 5 subjects received 2×10^{13} gc/kg	Enrolment complete Subjects dosed: 10 Interim CSR available (52 weeks) Study completed Subjects have been enrolled in extension Study CT-AMT-060-04 Final CSR (5 years): 06 January 2022	• FIX activity levels • Bleeding episodes • FIX replacement therapy • SF-36 QoL scores
CT-AMT-061-01: Phase 2b, open-label, single-dose, single-arm, multicentre study to confirm the FIX activity level of the serotype 5 AAV vector containing the Padua variant of a codon-optimized human FIX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult	Etranacogene dezaparvovec 3 subjects received 2×10^{13} gc/kg	Enrolment complete Subjects dosed: 3 Interim Report: 31 August 2020 (52 weeks) Interim CSR (52 weeks): 31 August 2020 Interim CSR (2.5 years): 07 December 2021	• FIX activity levels and FIX protein concentration • FIX replacement therapy • Bleeding episodes • PROs • HJHS

subjects with severe or moderately severe haemophilia B Sites and countries: 1 (US) Subjects require 52 weeks of Post-treatment Follow-up, followed by 4 years of LTFU		Final CSR expected: December 2023	
CT-AMT-061-02 (HOPE-B): Phase 3, open-label, single-dose, multicentre multinational study investigating a serotype 5 AAV vector containing the Padua variant of a codon-optimized human FIX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe haemophilia B Objective: To demonstrate noninferiority of etranacogene dezaparvovec (2×10^{13} gc/kg) during the 52 weeks of stable FIX expression (Months 6 to 18) after treatment, compared with standard of care continuous routine FIX prophylaxis during the Lead-in Phase, as measured by ABR. This study has a Screening Period, a Lead-in Phase, a Treatment plus Post-treatment Follow-up	Etranacogene dezaparvovec 53 subjects received 2×10^{13} gc/kg 1 subject received approximately 10% of the 2×10^{13} gc/kg dose	FPFV: 27 June 2018 Enrolment complete Screened: 75 Screen failures: 8 Entered the Lead-in Phase: 67 Discontinued during the Lead-in: 13 Treated: 54 Completed treatment (full dose): 53 Discontinued during follow-up: 1 Completed 18-months follow-up: 53 LPLV (18 months after dosing): 18 September 2021 18 months CSR: 21 February 2022 24 months DLP: 21 April 2022 LPLV (LTFU) expected: 20 March 2025 Final CSR expected: 30 July 2025	• Bleeding episodes • FIX activity levels and FIX protein concentration • FIX replacement therapy • PROs • HJHS • PROBE • Musculoskeletal ultrasound

Period, and a Long-term Follow-up Period. Sites: 29, countries: 9 Subjects require 1 year of Post-treatment Follow-up, followed by 4 years of LTFU			
CT-AMT-060-04: A phase 1/2b extension study assessing the long-term safety and efficacy of an AAV vector containing a codon-optimized human FIX gene (AAV5-hFIX) previously administered to adult subjects with severe or moderately severe haemophilia B during the CT-AMT-060-01 phase 1/2 study. Sites: 6, countries: 2	AMT-060	Number of subjects: 9/10 subjects previously treated in Study CT-AMT-060-01 (1 subject died after completion of Study CT-AMT-060-01) FPFV: 18 March 2021 LPLV expected: 01 May 2026 CSR expected: September 2026	• FIX activity • FIX replacement therapy • ABR (FIX-requiring) • Procedures (including major and minor surgery) • SF-36 and EQ-5D-5L • HJHS

AAV = adeno-associated virus; AAV5 = adeno-associated virus serotype 5; AAV5-hFIXco-Padua = recombinant adeno-associated viral vector containing a codon-optimized Padua derivative of human coagulation factor IX cDNA; ABR = annualized bleeding rate; cDNA = complementary DNA; CSR = Clinical Study Report; DE = Germany, DK = Denmark; DLP = data lock point; EQ-5D-5L = EuroQol-5 dimensions-5 levels; FIX = factor IX; FPFV = first patient first visit; gc = genome copies; hFIX = human factor IX; HJHS = Hemophilia Joint Health Score; LPLV = last patient last visit; LTFU = Long-term Follow up; NL = The Netherlands; No = number; PRO = Patient Reported Outcome; PROBE = Patient Reported Outcomes, Burdens, and Experiences; QoL = quality of life; SF-36 = Short-Form 36; US = United States.

The clinical development programme was initiated with AMT-060 (AAV5-hFIXco), the predecessor of etranacogene dezaparvovec.

AMT-060 was investigated at 2 dose levels in the first-in-human phase **1/2 Study CT-AMT-060-01**. Treatment was safe and well tolerated in both the low and high-dose cohorts. All 10 subjects converted to a mild or moderate haemophilia B phenotype and 9 out of 10 subjects achieved discontinuation of routine FIX replacement therapy. At 5 years of follow-up, all subjects continued to stably express FIX (mean FIX ranged between 2.8 to 10.7%) and showed a clinically relevant reduction in annualised bleeding rate (ABR) for total and spontaneous bleeding episodes.

Following Health Authority interaction, and in order to increase FIX levels further towards a normal FIX range (≥ 40%), the drug product was modified to express the naturally occurring hFIX-Padua variant (AAV5-hFIXco-Padua; etranacogene dezaparvovec).

Clinical development with etranacogene dezaparvovec continued by enrolling 57 subjects into Study **CT-AMT-061-01** (N = 3) and Study **CT-AMT-061-02** (N = 54) regardless of the subjects' preexisting anti-AAV5 neutralizing antibody (nAb) titres.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

AMT-061 (etranacogene dezaparvovec) is a viral gene therapy product to be administered intravenously. Conventional pharmacokinetic analyses do not apply to products that form *in vivo*, and therefore no clinical studies have been conducted to investigate the classical aspects of absorption, metabolism or excretion of AMT-061. The kinetics of AMT-061-derived FIX activity and FIX protein concentrations in plasma, the specific activity of the AMT-061-derived FIX, the distribution of AMT-061 vector DNA in blood, and the shedding of AMT-061 vector DNA in secreta and excreta are discussed under Clinical efficacy and Clinical safety, respectively.

Factor IX (FIX) activity was measured by two methods: the one-stage clotting assay and the chromogenic/amidolytic assay.

In the one-stage aPTT clotting assay, incubation of the plasma sample with an optimal quantity of phospholipid, a negatively charged activator and buffer initiate the activation of the intrinsic coagulation pathway. After incubation at 37 °C for a specific period of time calcium is added to trigger the coagulation process and the time required for clot formation is measured at a wavelength of 671 nm. The clotting time in seconds (s) is then converted to a % activity of FIX based on the calibration curve, which consists of human plasma with a certified FIX level.

The following parameters were validated: calibration curve fit, carry over, precision and accuracy, lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ), measurement range, drift, matrix effect (effect of haemolysis, lipemia and icterus), FIX stability in plasma at ambient temperature, FIX freeze/thaw stability in plasma in low temperature freezer, and FIX stability in plasma in low temperature freezer. All validation criteria were met. The measurement range was established between 0.6 – 110.3 % FIX activity. The method was shown to perform well on samples from treated patients.

The FIX chromogenic assay uses the colorimetric principle of measuring absorbance of light by the solution in a cuvette. The amount of light that reaches the photo-detector is converted into an electrical signal that is proportional to enzymatic activity. The method for assessing FIX activity uses the BIOPHEN chromogenic kit produced by Hyphen Biomed. The following parameters were validated: calibration curve fit, carry over, precision and accuracy, lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ), measurement range, drift, matrix effect (effect of haemolysis, lipemia and icterus), FIX stability in plasma at ambient temperature, FIX freeze/thaw stability in plasma in low temperature freezer and FIX stability in plasma in low temperature freezer. The method was successfully validated and performed well on samples obtained from treated patients. The measurement range for this method was defined as 0.8 - 109.3 % FIX activity.

The kinetics of FIX activity, FIX protein, clearance of vector DNA and immunogenicity, along with safety and tolerability of AMT-060, were evaluated in the first-in-human phase 1/2 study (Study CT-AMT-060-01) in subjects with moderately severe or severe haemophilia B. The kinetics of FIX activity, FIX protein, clearance of vector DNA, immunogenicity, along with efficacy and safety, were evaluated in Studies CT-AMT-061-01 and CT-AMT-061-02.

In addition to these individual studies, analyses were performed for the impact of both intrinsic factors and extrinsic factors and immunogenicity on FIX activity, using data from Study CT-AMT-061-02. Analyses of durability of FIX activity and FIX protein expression of etranacogene dezaparvovec were performed using combined data from Studies CT-AMT-061-01 and CT-AMT-061-02 with supportive evidence from Study CT-AMT-060-01.

Biodistribution of AMT-061 was tested in human blood and semen by Q-PCR. The method was validated for AMT-060. The sequence of primers and probe was not shown. For control plasmid (inhibitory control), full map and sequence, with primers and probe highlighted was provided. Acceptable recoveries were demonstrated from all matrices tested (blood, urine, semen, nasal secretions, faeces and saliva). LOD was set at 10 copies per reaction and LLOQ at 100 copies per reaction. None of the extracts showed inhibitory properties. Validation is considered appropriate.

Results of tests carried out in blood and semen of participants in the Phase III clinical trial were presented. A brief description of the method used, including the sequence of primers and probes, was submitted. Although no details on the location of primers and probes were provided, this is not considered critical as positive results were reported. The method was validated with AMT-060 but results of this study show that the method is suitable for detecting AMT-061 as well.

2.6.2.2. Pharmacodynamics

Etranacogene dezaparvovec is a somatic gene therapy product that aims to deliver a nucleic acid expression cassette capable of driving expression and synthesis of functional FIX to the liver of patients with haemophilia B. One-time treatment with etranacogene dezaparvovec allows the patient to continuously produce functional human FIX (hFIX)-Padua protein at levels which modify the severity of their haemophilia B disease.

Etranacogene dezaparvovec consists of a hFIX-Padua coding sequence (hFIXco-Padua) expression cassette, which is packaged within a recombinant adeno-associated virus serotype 5 vector (rAAV5). Codon-optimisation introduced silent nucleotide changes in the hFIXco, which may improve messenger ribonucleic acid stability and FIX gene expression, while not changing the resulting amino acid sequence.

The naturally occurring hFIX-Padua variant encoded by the gene expression cassette of etranacogene dezaparvovec differs from the wild-type hFIX protein by a single amino acid substitution of the mature protein (Arg [AAG] → Leu [CTG] at position 338 [R338L]), which increases FIX activity 6 to 8-fold.

The hFIX coding sequence is flanked upstream by the liver-specific promoter-1 (LP-1), driving liver-specific transgene expression and downstream by the SV40 polyA (transcription termination, polyadenylation). Between the LP-1 promoter and the hFIX / hFIX-Padua coding sequence is a SV40 intron (to promote transgene expression). The entire expression cassette is flanked by inverted terminal repeats.

The PD effect of treatment with Hemgenix consists of the expression of functional FIX protein by transduced liver cells, the activity of which can be measured in the plasma. FIX activity is the primary efficacy endpoint of the phase 2b study and a secondary efficacy endpoint of the pivotal phase 3 trial, therefore those outcomes are discussed in the efficacy part of this assessment report.

2.6.3. Discussion on clinical pharmacology

The Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014) specifies that classical pharmacokinetic studies based on absorption,

distribution, metabolism and excretion (ADME) studies are usually not required for GTMPs. Therefore, the lack of clinical pharmacology studies in this dossier is acceptable. The same guideline further clarifies that on a case by case basis, pharmacokinetics studies need to be carried out depending on the specific GTMPs, e.g. if the gene product is a protein excreted in the blood circulation. Plasma levels of the induced FIX activity are defined as the primary endpoint of phase 2b study CT-AMT-061-01 and as a secondary endpoint of the pivotal study CT-AMT-061-02 and are discussed in the efficacy part of this assessment report.

Vector DNA biodistribution and shedding are discussed in the safety part of this assessment report.

The pharmacodynamic effect of Hemgenix is the induction of relevant plasma levels of FIX, which restore the coagulatory ability of the patient's blood. Endpoints illustrating this PD effect are plasma levels of FIX, which are defined as a primary or secondary endpoint in the submitted clinical trials. Despite these laboratory values being of interest as established surrogate endpoints of efficacy in haemophilia trials, the main aim of gene therapy with Hemgenix is to provide patients with the freedom from bleeding events and also freedom from regular prophylactic and/or therapeutic infusions of external factor IX. Therefore, the endpoints with the most clinical relevance are the annualised bleeding rate, annualised factor IX consumption and annualised FIX infusion rate, which are all discussed in the clinical efficacy part of this assessment report.

2.6.4. Conclusions on clinical pharmacology

The product can be approved on pharmacology grounds.

The CHMP endorses the CAT assessment regarding the conclusions on the clinical pharmacology aspects as described above.

2.6.5. Clinical efficacy

The main evidence for efficacy and safety derives from the phase 2b trial CT-AMT-061-01 and the pivotal trial CT-AMT-061-02, in which a combined 57 subjects were enrolled. 2.5 years of follow-up is available for the phase 2 study, and 1.5 years for the pivotal study. With the responses to the D120 LoQ, the applicant submitted efficacy data up to month 36 of CT-AMT-061-01 and up to month 24 for pivotal trial AMT-061-02.

2.6.5.1. Dose response study

CT-AMT-061-01

Phase IIb, open-label, single-dose, single-arm, multi-centre trial to confirm the factor IX activity level of the serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimised human factor IX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe haemophilia B

CT-AMT-061-01 is an ongoing Phase IIb trial consisting of a screening phase, a treatment plus post-treatment follow-up phase, and a long-term follow-up phase. After a maximum 6-week screening period, 3 eligible subjects received a single IV dose of 2×10^{13} gc/kg AMT-061. Subjects were monitored for tolerance to AMT-061 and detection of immediate AEs for 24 hours (overnight stay) after dosing. The dosing of the subjects was separated by a minimum of 14 calendar days to allow for subject safety monitoring and to ensure appropriate action could be taken in case any acute reactions were observed.

Diagnosis and Main Criteria for Inclusion: Males 18 years or older with known severe or moderately severe FIX deficiency (1-2% of normal circulating FIX, inclusive and a severe bleeding phenotype) with at least 20 exposure days to FIX products could enrol. Patients could be on either prophylaxis or on-demand therapy, but patients on on-demand therapy must have a history of frequent bleeding (4 or more bleeding episodes in the last 12 months) or chronic haemophilic arthropathy (pain, joint destruction, and loss of range of motion) in 1 or more joints. Patients with a history of FIX inhibitors, HIV-positive patients with CD4+ counts ≥200/μL, subjects who had ALT, AST or total bilirubin >2 times the upper limit of normal, patients with active infection with hepatitis B or C virus and patients with a history of hepatitis B or C exposure, currently controlled by antiviral therapy, were excluded from participation in the clinical trial.

Primary Efficacy Variables/Endpoints:

Factor IX activity level at six weeks after dosing.

Secondary Efficacy Variables/Endpoints:

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

Exploratory efficacy endpoints include joint health and QoL scores, correlation between AAV5 neutralizing antibodies titres and factor IX activity levels, and factor IX-protein-to-activity ratio in subjects without residual expression of non-functional factor IX protein.

Blood samples for determination of *endogenous FIX activity* and *FIX protein* were collected and assessed at the central and/or local laboratory. Central laboratory results for FIX activity were used in the analyses. Local laboratory results for FIX activity may have been used for local monitoring of subjects, to assess the potential need for exogenous FIX, but not to assess treatment outcome. Descriptive statistics and plots only display uncontaminated results, i.e., factor IX activity levels that were not affected by exogenous factor IX use during the trial. The required wash out period (in order to consider a factor IX activity level to be "unaffected" – i.e., "uncontaminated") was 10 days.

From the Screening Visit until Week 52, subjects recorded the use of all *prophylactic and on-demand FIX replacement therapy* in an e-diary. In the long-term follow-up phase, subjects record information of FIX replacement therapy in a study-specific paper diary.

From the Screening Visit until Week 52, subjects recorded information of *bleeding episodes* in an e-diary. The Investigator assessed each bleeding episode reported in the e-diary as soon as possible within 72 hours after it was reported, and recorded the outcome of the bleeding episode. In the long-term follow-up phase, subjects recorded information of bleeding episodes in a study-specific paper diary, which they brought to each visit. Information that was new since the previous visit was collected by site staff. Between visits, subjects also contacted the site immediately in case of an experienced bleed and provided relevant information.

Patient Population: This study was initiated on 24 July 2018 (first subject's informed consent date) and occurred at 4 study centres in the United States. A total of 3 subjects were screened and treated in the

study. All 3 subjects completed 2.5 years (30 months) of follow-up post-AMT-061 administration by 18 March 2021. All subjects remain in the long-term follow-up period of the study at the time of the data cut-off. Study subjects were male and, at screening, were 43, 47, and 50 years old. Two subjects were African American (Black) and 1 subject was Caucasian (White). Two suffered from severe haemophilia B and one from moderately severe HB.

Subjects had 1, 3, or 5 bleeding episodes in the year before screening, which were all spontaneous and varied in severity from mild to severe. No subject had any target joints at screening (i.e., a joint into which they had bled at least 3 times in the 6 previous months). In the 12 months before the Screening Visit, all 3 subjects used prophylactic and on-demand FIX replacement therapy and all subjects had > 150 days of exposure to FIX.

All 3 subjects had a prior Hepatitis C infection, and 2 subjects had a controlled HIV infection.

Efficacy Outcomes

Primary Efficacy Endpoint

FIX Activity at Week 6

At Week 6, mean ± SD uncontaminated FIX activity level was 30.6 ± 6.97% of normal measured by the one-stage (aPTT-based) assay. Individual FIX activity levels achieved by each subject at Week 6 were 23.9%, 30.0%, and 37.8%. This is considerably higher than values achieved in the Phase I study CT-AMT-060-01, where mean activity was approximately 7.5% with a dose of 2×10^{13} gc/kg of an AAV vector carrying the wild-type FIX DNA.

Secondary Efficacy Endpoints

Factor IX Activity Levels at Week 52

At Week 52, mean ± SD uncontaminated FIX activity level was 40.8 ± 9.45% of normal measured by the one-stage (aPTT-based) assay. Individual FIX activity levels achieved by each subject at Week 52 were 31.3%, 40.8%, and 50.2%

Factor IX Activity Levels Post-AMT-061 Administration

Factor IX activity levels measured by the one-stage (aPTT-based) assay increased for all 3 subjects to clinically relevant levels following 1-time administration of AMT-061. At Baseline, uncontaminated mean FIX activity was 5.1%, based on data for 1 subject. The other 2 subjects did not have a baseline FIX assessment that was considered uncontaminated (i.e., assessments post-AMT-061 administration that were more than 5 half-lives of exogenous FIX use), and therefore were not included in this calculation. Mean ± SD FIX activity was 23.4 ± 1.04% of normal at Week 3, increasing to 30.6 ± 6.97% of normal at Week 6, and 40.8 ± 9.45% of normal at Week 52. At Month 18, Month 24, and Month 30, mean ± SD FIX activity was 47.0 ± 12.66%, 44.2 ± 7.66%, and 50.0 ± 11.40% of normal, respectively. At Month 36, 3 years post-AMT-061 administration, uncontaminated samples were available for 2 subjects and demonstrated that FIX activity levels continued to be elevated, at 32.3% and 41.5%, respectively.

Annualised Bleeding Rates and Bleeding Episodes

The average ABR for the 3 subjects, calculated as the total number of bleeding episodes divided by the time (in years) at risk, was 0.27 over the period of 2.5 years (30 months) of follow-up. The ABRs for spontaneous and traumatic bleeding episodes over 2.5 years (30 months) were both 0.14. The average ABR for the 3 subjects, calculated as the total number of bleeding episodes divided by the time (in years) at risk, was 0.22 over the period of 3 years (36 months) of follow-up. The ABRs for spontaneous and traumatic bleeding episodes over 3 years (36 months) were both 0.11. There were

no bleeding episodes between 2.5 and 3 years of follow-up (both bleeding episodes occurred in the first 18 months post-AMT-061 administration).

As this trial had no run-in phase specified in the protocol, a comparison to meaningful pre-treatment data is not possible.

Use of Factor IX Replacement Therapy

The annualised mean FIX replacement use was 306,204.9 IU/year in the 1 year prior to screening, 260,285.8 IU/year in the 30 days prior to screening, and 299,330.7 IU/year during screening (Table 2). During the screening period, the annualised mean FIX replacement use was 250,726.5; 266,583.8; and 380,681.8 IU/year for the 3 subjects. Subjects used extended half-life products during the screening period.

The annualised mean FIX use was 1220.4 IU/year over 2.5 years (30 months) of follow-up post-AMT-061 administration and was 689.1 IU/year for the period following discontinuation of routine prophylaxis (the post-continuous prophylaxis period; Table 2). All subjects discontinued use of routine prophylaxis FIX use between 1 and 4 days post-AMT-061 administration. Over 3 years (36 months) of follow-up, the annualised mean FIX use was 1157.2 IU/year post-AMT-061 administration and was 714.6 IU/year for the period following discontinuation of routine prophylaxis (the post-continuous prophylaxis period.

Exogenous FIX consumption during the post-treatment phase was low, but as this trial had no run-in phase specified in the protocol, a comparison to meaningful pre-treatment data is not possible.

Exploratory Efficacy Measure

Factor IX Activity Levels – Chromogenic Assay

At Baseline, Week 6, Week 52, and Month 30, mean ± SD uncontaminated FIX activity measured by the chromogenic assay was 2.7%, 17.5 ± 3.64%, 22.2 ± 5.98%, and 22.3 ± 5.90%, respectively (Table 3), compared to 5.1%, 30.6 ± 6.97, 40.8 ± 9.45%, and 50.0 ± 11.40%, respectively, measured by the one-stage (aPTT-based) assay. Uncontaminated factor IX activity measured using the chromogenic assay was approximately half of what was measured using the one-stage assay (mean ratio ranged between 0.4431 and 0.6337).

A discrepancy was noted if FIX activity is measured with the one-stage and the chromogenic assay. The chromogenic assay returns approximately half the values observed with the one-stage assay. Therefore the correlation of values returned by both assays has to be investigated with a special emphasis on high and low values of FIX activity. The discrepancy has to be mentioned in section 4.4 of the SmPC and a conversion factor has to be provided to allow the treating physician a meaningful monitoring of factor levels with either assay. Furthermore, when using an *in vitro* thromboplastin time (aPTT)-based one stage clotting assay for determining factor IX activity in patients' blood samples, plasma factor IX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay. This is of importance particularly when changing the laboratory and/or reagents used in the assay. The applicant is therefore asked to clarify which reagents/reference standards have been used to monitor patients' FIX levels in the clinical trial programme and to add a description of these assays to the SmPC in the same section as above. In addition, the applicant was asked to clarify if the assays used for monitoring FIX levels in the clinical trial environment correspond to those widely available for routine clinical monitoring in the EU.

With their responses to the D120 LoQ, the applicant argued that a conversion factor between the two assay methods is not warranted due to the one-stage assay being the most commonly used method to monitor FIX activity levels in clinical practice. Furthermore, the mean ratio of FIX activity by chromogenic assay to one-stage (aPTT-based) assay ranged from 0.408 to 0.547 across post-dose

time-points up to Month 24 and, accordingly, did not allow for a robust single conversion factor between these 2 assays.

However, as the applicant also mentions, the chromogenic assay is used in cases when a clinical bleeding phenotype appears to be discrepant from the FIX activity as measured by the one-stage assay. Therefore, even if it is not possible to provide a meaningful conversion factor, the fact that the two assay systems return discrepant values when monitoring FIX activity induced by AMT-061 treatment is brought to the attention of the treating physician with a warning statement in section 4.4 of the SmPC.

Of the 33 investigator sites used in pivotal trial AMT-061-02, 13 were in the EU (BE, DK, IT, NL, DE, SE and IE). The central laboratory used to determine FIX activity was Unilabs a.s. Bioanalytical Solutions in Copenhagen, Denmark. The central laboratory used the validated HemoSiL SynthASiL (IL) one stage assay platform while the investigator sites used HemoSiL SynthASiL (IL), PTT Automate (Stago), Actin FS (Siemens) and Actin FSL (Siemens) one stage assay platforms for local FIX activity determination. The applicant has provied a correlation analysis of FIX activity values determined at the central and local labs and an analysis of the ratio of FIX activity measured locally and centrally. Both analyses support the notion that FIX activity can be determined in a reproducible way using the four different assay systems. The one-stage assays used in the pivotal trial correspond to the assay systems most commonly used worldwide [Sommer et al, Int J Lab Hematol, 2020; 42:350-58], in addition, as described above, the central lab as well as 13 investigator sites were in the EU and used representative assay platforms.

Factor IX Activity Levels and Neutralizing Antibodies

All subjects had pre-existing nAbs to AAV5 at the Screening Visit, defined as having a titre ≥7. Titres were 25.2, 43.8, and 47.6 at the Screening Visit, and 19.5, 22.1, and 33.0 at the Baseline Visit prior to administration of AMT-061. By Week 2, titres were >36,450.0 (the upper limit of quantification) for all subjects and titres remained >36450.0 through to Month 24. With pre-existing nAbs, subjects still achieved a mean FIX activity of approximately 30.6% by Week 6, 40.8% at Week 52, and 50.0% at Month 30.

All three subjects displayed low titre neutralising antibodies against AAV at baseline. Interestingly, the subject with the highest titre consistently had the lowest FIX activity levels. However, the low number of subjects precludes meaningful conclusions from these data.

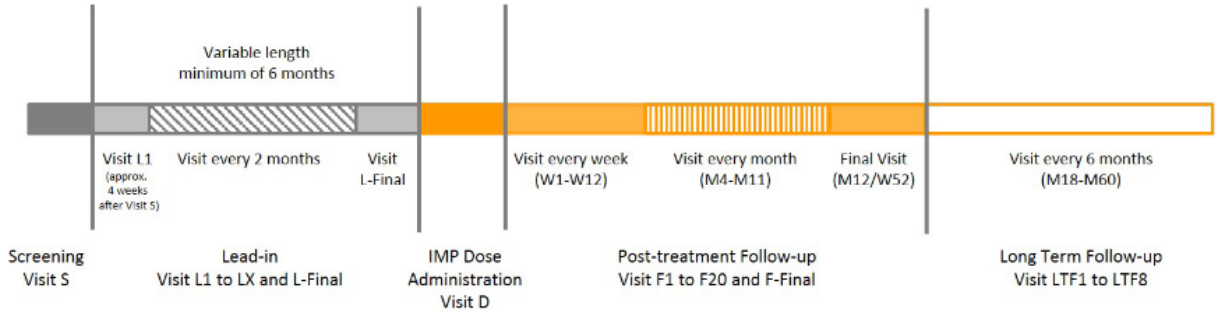
2.6.5.2. Main study

CT-AMT-061-02 (HOPE B)

Phase III, open-label, single-dose, multi-centre multinational trial investigating a serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimised human Factor IX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe haemophilia B

CT-AMT-061-02 (Health Outcomes with Padua Gene; Evaluation in Haemophilia B [HOPE B]) is an ongoing open-label, single-dose, multi-centre, multinational trial, with a screening phase/period, a lead-in phase/period, a treatment plus a post-treatment follow-up phase/period, and a long-term follow-up phase/period.

Figure 1. Study Design Diagram CT-AMT-061-02



Abbreviations: D = dosing; F = post-treatment follow-up; IMP = investigational medicinal product; L = lead-in; LTF = long-term follow-up; M = Month; S = screening; W = week.

During the lead-in phase, which lasted a minimum of 26 weeks, subjects recorded their use of FIX replacement therapy and bleeding episodes in their dedicated e-diary in order to provide a baseline of bleeding event frequency and FIX consumption.

Methods

- **Study Participants**

Main inclusion criteria:

Adult subjects with congenital haemophilia B with known severe or moderately severe FIX deficiency (≤ 2% of normal circulating FIX) for which the subject was on continuous routine FIX prophylaxis and had >150 previous exposure days of treatment with FIX protein.

Main exclusion criteria:

Subjects with a history of FIX inhibitors, ALT >2 times ULN, AST >2 times ULN, Total bilirubin >2 times ULN (except if caused by Gilbert disease), Alkaline phosphatase (ALP) >2 times ULN, Creatinine >2 times ULN; Positive HIV serological test at screening and Visit L-Final, not controlled with anti-viral therapy as shown by CD4+ counts ≤200/ μ L (based on central laboratory results); Hepatitis B or C infection with the following criteria present at screening: Currently receiving antiviral therapy for this/these infection(s) and/or positive for any of the following - Hepatitis B surface antigen (HBsAg), except if in the opinion of the Investigator this was due to a previous hepatitis B vaccination rather than active hepatitis B infection - Hepatitis B virus (HBV) DNA - Hepatitis C virus (HCV) ribonucleic acid (RNA); Known significant medical condition that may have significantly impacted the intended transduction of the vector and/or expression and activity of the protein

- **Treatments**

Subjects were planned to receive a single IV infusion of 2×10^{13} gc/kg AMT-061.

The pharmaceutical form of AMT-061 was a solution for IV infusion.

AMT-061 was formulated as a sterile solution at a concentration of approximately 1×10^{13} gc/mL in Phosphate Buffer Saline (pH 7.2) with 5% (w/w) sucrose and 0.02% (v/v) polysorbate 20. The IMP was supplied in 10 R clear glass type I vials filled with approximately 10 mL of AMT-061. Each vial contained an extractable volume of at least 10 mL.

The AMT-061 product remained in its original secondary packaging and was stored frozen at ≤ -65° C at all times prior to use. AMT-061 was stable at ≤-65° C for at least 24 months and for a maximum of 24 hours at room temperature (as of first vial break and until start of administration). Each vial of AMT-061 was labelled with the product name, batch number, vial number, product concentration,

manufacturing date, and storage conditions. Sites were instructed to protect prepared infusion bags from light during room temperature storage.

The reference therapy was the prophylaxis FIX replacement therapy used during the lead-in phase prior to treatment with AMT-061.

- **Objectives**

Primary Objective

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

Secondary Objectives

The secondary objective was to demonstrate additional efficacy and safety aspects of systemic administration of AMT-061.

Secondary efficacy objectives were focused on investigating the effect of 2×10^{13} gc/kg AMT-061 on the following:

- Endogenous FIX activity 6 months after a single AMT-061 treatment
- Endogenous FIX activity 12 months after a single AMT-061 treatment
- Endogenous FIX activity 18 months after a single AMT-061 treatment
- Annualised consumption of FIX replacement therapy
- Annualised infusion rate of FIX replacement therapy
- Discontinuation of previous continuous routine prophylaxis
- Trough FIX activity
- Prevention of bleedings (comparison for superiority)
- Prevention of spontaneous bleeding
- Prevention of joint bleeding
- Estimated ABR – during the 52 weeks following stable FIX expression (6 to 18 months) – as a function of pre-investigational medicinal product (IMP) anti-AAV5 antibody titres using the luciferase based nAb assay (as a "correlation" analysis)
- Correlation of pre-IMP anti-AAV5 antibody titres using the luciferase based nAb assay on FIX activity levels after AMT-061 dosing
- Occurrence and resolution of target joints
- Proportion of subjects with zero bleeding episodes during the 52 weeks following stable FIX expression (6 to 18 months) after AMT-061 dosing
- International Physical Activity Questionnaire (iPAQ)
- EuroQol-5 dimensions-5 levels (EQ-5D-5L) Visual Analog Scale (VAS)

Exploratory Objectives

Exploratory efficacy objectives investigated the effect of AMT-061 on the following:

- Factor IX protein levels during the 18 months following AMT-061 dosing
- Haemophilia Joint Health Score (HJHS) scores
- Other Patient Reported Outcome (PRO) questionnaires: Work Productivity and Activity Impairment Questionnaire (WPAI), Brief Pain Inventory (BPI), Haemophilia Activities List (HAL), and Haemophilia Quality of Life Questionnaire for Adults (Hem-A-QoL) during the lead-in phase (prophylaxis) and during the 12 months following AMT-061 dosing
- Estimated ABR over time as a function of mean FIX activity (as a "correlation" analysis) over the 18 month post-AMT-061 treatment follow-up
- Rate of traumatic bleeding events during the 52 weeks following stable FIX expression (6 to 18 months) post-treatment follow-up compared to the lead-in phase
- Subgroup analyses will be carried out for the following endpoints:
  - Endogenous FIX activity at 18 months
  - Annualised consumption of FIX replacement therapy, excluding replacement for invasive procedures
  - Annualised infusion rate of FIX replacement therapy
  - ABR comparison between AMT-061 and FIX prophylaxis
  - Comparison of the percentage of subjects with trough FIX activity <12% of normal between the lead-in phase and after treatment with AMT-061 over the 52 weeks following stable FIX expression (6 to 18 months)
  - Proportion of subjects remaining free of previous prescribed continuous routine prophylaxis.
  - All efficacy endpoints (as exploratory endpoints) at 2, 3, 4, and 5 years after AMT-061 dosing

- **Outcomes/endpoints**

<u>Primary efficacy endpoint</u>

- Annualised bleeding rate comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment)

<u>Secondary efficacy endpoints</u>

- Endogenous FIX activity at 6 months after AMT-061 dosing
- Endogenous FIX activity at 12 months after AMT-061 dosing
- Endogenous FIX activity at 18 months after AMT-061 dosing
- Annualised consumption of FIX replacement therapy during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment), excluding FIX replacement for invasive procedures, compared to the lead-in phase
- Annualised infusion rate of FIX replacement therapy during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment), excluding FIX 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 FIX expression (Months 6 to 18 post-treatment)
- Comparison of the percentage of subjects with trough FIX activity <12% of normal between the lead-in phase and after treatment with AMT-061 over the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment)
- Annualised bleeding rate comparison between AMT-061 and prophylaxis for superiority between the lead-in phase and the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment)
- Rate of spontaneous bleeding episodes during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) compared to the lead-in phase
- Rate of joint bleeding episodes during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) compared to the lead-in phase
- Estimated ABR – during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) – as a function of pre-IMP anti-AAV5 antibody titres using the luciferase based NAb assay (as a "correlation" analysis)
- Correlation of FIX activity levels during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) with pre-IMP anti-AAV5 antibody titres using the luciferase based NAb assay
- Occurrence of (and resolution of) new target joints during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) and resolution of pre-existing target joints following AMT-061 dosing
- Proportion of subjects with zero bleeding episodes during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment)
- Patient reported outcome (PRO) questionnaire scores from the iPAQ (total physical activity score) during the 12 months following AMT-061 dosing compared with the lead-in phase
- PRO questionnaire scores from the EQ-5D-5L VAS score during the 12 months following AMT-061 dosing compared with the lead-in phase

Exploratory endpoints

- FIX protein levels during the 18 months following AMT-061 dosing
- HJHS scores during the lead-in phase (prophylaxis) and during the 12 months following AMT-061 dosing
- Other PRO questionnaires: WPAI, BPI, HAL, and Hem-A-QoL questionnaire scores during the lead-in phase (prophylaxis) and during the 12 months following AMT-061 dosing
- EQ-5D-5L index scores during the lead-in phase (prophylaxis) and during the 12 months following AMT-061 dosing
- Estimated ABR as a function of mean FIX activity (as a "correlation" analysis) over the 18 months post-AMT-061 treatment follow-up
- Rate of traumatic bleeding episodes during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) compared to the lead-in phase
- Subgroup analyses were carried out for the following endpoints:

o Endogenous FIX activity at Month 18

o Annualised consumption of FIX replacement therapy during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment), excluding replacement for invasive procedures, compared to the lead-in phase

o Annualised infusion rate of FIX replacement therapy during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment), excluding replacement for invasive procedures, compared to the lead-in phase

o ABR comparison between AMT-061 during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) and FIX prophylaxis (during the lead-in phase)

o Comparison of the percentage of subjects with trough FIX activity <12% of normal between the lead-in phase and after treatment with AMT-061 during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment)

o Proportion of subjects remaining free of previous prescribed continuous routine prophylaxis during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment)

- **Sample size**

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. For the secondary statistical analyses of factor IX activity at 6, 12, and 18 months, assuming a mean of 30.6 percent of normal (as observed at 6 weeks in study CT-AMT-061-01) and assuming a standard deviation of 6.97 (as observed at 6 weeks in study CT-AMT-061-01), assuming conservatively that the baseline factor IX activity is 2%, and assuming that the sample size is 50 subjects, for a one-sample t-test at the 0.025 one-sided level of significance to test whether the change from baseline is > 0, the statistical power is > 99%. Alternatively, assuming that the standard deviation is 6.95, which is half of the range of factor IX activity values (23.9 to 37.8) observed at 6 weeks in study CTAMT- 061-01, the statistical power is still > 99%. The nQuery Advisor software was employed for this power calculation.

- **Randomisation and Blinding (masking)**

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

- **Statistical methods**

Analysis population

Screen Failures
The screen failure population included all subjects who were screened but never entered the lead-in period.

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

Safety Population
The lead-in safety population consisted of all subjects who are enrolled into the lead-in period. The post-treatment safety population consisted of all subjects who receive AMT-061, irrespective of any protocol deviations. Period-specific safety tabulations used the period-specific safety population for the "N" and denominator (for percentages). The safety population consisted of all subjects who are in either the lead-in safety population or the post-treatment safety population.

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

Per-Protocol Population

The PP population included all subjects from the FAS population who adhered to a stable and adequate prophylaxis use during the lead-in phase, who completed at least 18 months of efficacy assessments (52 weeks after achieving stable FIX expression) for the 18-month (data cut) analysis who completed at least a full year of efficacy assessments for the 12-month (data cut) analysis, or who completed at least 6 months of efficacy assessments for the 6-month (data cut) analysis, and who had no major protocol deviations that impact the interpretation of efficacy. The PP population was used for sensitivity analyses. Protocol deviations that impacted the interpretation of efficacy included the unwillingness to discontinue continuous prophylaxis use after receipt of AMT-061.

Primary Analysis

The primary efficacy endpoint was defined as follows:

Annualised bleeding rate (ABR) comparison between AMT-061 and prophylaxis for non-inferiority between the 52 weeks following stable FIX expression (6-18 months) post treatment (AMT-061) follow-up and the lead-in phase

ABR was determined for the lead-in period and post-treatment period (for the 52 weeks following stable FIX expression [6-18 months]). Analysis of the number of reported bleeding events was performed using a repeated measures generalised 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 was employed. If the model fails to converge, then a compound symmetry covariance structure is used. The model included the treatment (i.e. period) as a categorical variable. If convergence was not attained, then initial parameter estimates were provided. The estimated rate ratio and one-sided 97.5% Wald CI and the corresponding p-value was determined. The upper limit of the resultant CI of the rate ratio was compared to the non-inferiority margin of 1.8. If the upper limit is less than 1.8, then non-inferiority is declared.

Several sensitivity analyses were performed:

- Primary analysis on PP population instead of FAS
- Including (not excluding) periods subsequent to exogenous factor IX use
- Bleeds treated with exogenous factor IX
- Cumulative responder analysis using subject-specific bleeding rates
- New and true bleeds
- New and true bleeds treated with exogenous factor IX
- Excluding periods contaminated by systemic corticosteroid exposure
- Optional zero-inflated negative binomial regression

Subgroup analysis

Subgroup analyses were carried out for the following endpoints (subgroups are defined below):

- Endogenous factor IX activity at month 18
- Annualised consumption of factor IX replacement therapy during the 52 weeks following stable FIX expression (6-18 months) post-treatment follow-up, excluding replacement for invasive procedures, compared to the lead-in phase
- Annualised infusion rate of factor IX replacement therapy during the 52 weeks following stable FIX expression (6-18 months) post-treatment follow-up, excluding replacement for invasive procedures, compared to the lead in phase
- ABR comparison between AMT-061 (during the 52 weeks following stable FIX expression [6-18 months] post-treatment follow-up) and factor IX prophylaxis (during the lead-in period)
- 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 FIX expression (6-18 months)
- Proportion of subjects remaining free of previous prescribed continuous routine prophylaxis during the 52 weeks following stable FIX expression (6-18 months) post-treatment follow-up.

Missing Data

Missing data was maintained as missing in the safety and efficacy datasets, unless specified otherwise.

If causality was missing for a TEAE, the TEAE was regarded as ‘Related’. If causality was missing for an AE with onset before administration of trial drug, the AE was regarded as 'Not related'. If the intensity was missing, the intensity of the AE was regarded as “Severe.” In the case where seriousness was missing, this should be queried. Seriousness cannot be imputed as 'Yes' by default, since this would affect the reconciliation between trial database and registry of SAEs.

After 18 months post-dose, efficacy and safety data were collected, and the data were locked and analysed. These data included all subject-specific Month 18 visits, as well as visits beyond Month 18 if they occurred (or the events/exposures began) before 31 August 2021. The results from this 18-month analysis are presented in this CSR.

Detailed methodology for the display, summary, and statistical analyses of the data collected in this study were documented in a SAP, dated 10 June 2021, prior to the data cutoff date.

Except where specified, all continuous variables were summarised with descriptive statistics (the number of non-missing values, mean, SD, median, minimum, maximum, quartiles [Q1 and Q3]) and all categorical variables were summarised with frequency counts and percentages, by treatment group. Data were presented by study phase as appropriate.

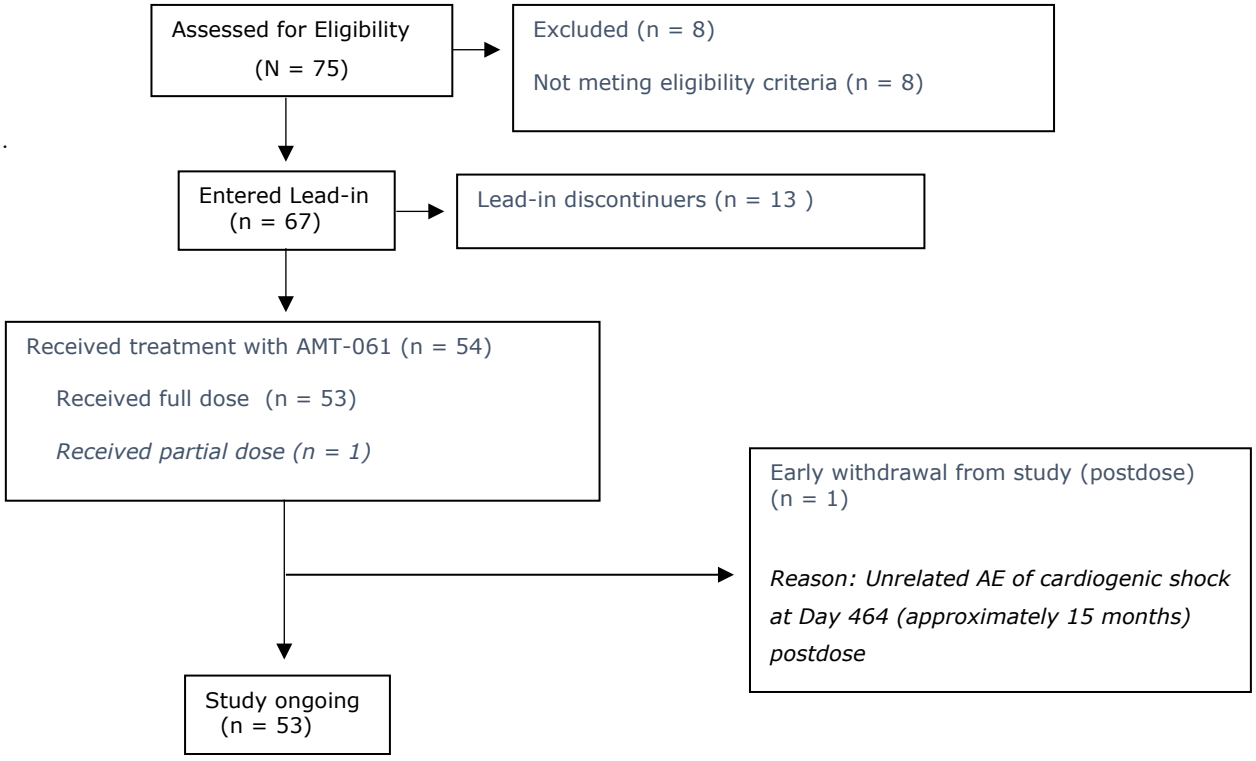
Hypothesis testing for the primary endpoint was carried out as a one-sided non-inferiority test with a non-inferiority margin of 1.8. Formal statistical testing of the efficacy endpoints (where performed), using a hierarchical approach, tested for superiority at a one-sided alpha level of 0.025.

Time-to-event data were summarised using the Kaplan-Meier method, as appropriate. Statistical analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC 27513).

Results

- **Participant flow**

Figure 2. Participant Flow Study CT-AMT-061-02



A total of 75 subjects were screened; 67 subjects were enrolled and were included in the Lead-in Safety Population. A total of 54 subjects received AMT-061 and were followed for efficacy and safety and included in the Post-treatment Safety Population.

- **Recruitment**

Study start date: 27 June 2018 (First subject's informed consent date)

The study is currently ongoing.

- **Conduct of the study**

Protocol amendments:

There were 6 global protocol amendments implemented during the trial.

In Amendment 1, it was specified that factor inhibitor testing at screening and Visit L-Final (exclusion criterion #2) would be based on local laboratory results, while all other screening laboratory tests (exclusion criteria #3, 4, 5, and 8) would be based on central laboratory results. Inclusion criterion #4 was updated to include subjects with >150 exposure days of treatment with FIX protein. The timing of assessments was refined. Additionally, a chromogenic assay for FIX activity was added; the timing and volume of blood draws was updated to integrate the new assay.

In Amendment 2, the number of study sites was increased to approximately 50. The dose of AMT-061 was established as 2×10^{13} gc/kg, reflecting results from the interim analysis of the dose confirmation study (CT-AMT-061-01). In this amendment, FibroScan™ was added as an assessment tool for liver health and it was specified that the Investigator and subject should discuss the importance of a healthy liver before and after receiving therapy. Advanced liver fibrosis and a known history of corticosteroid allergy were added as exclusion criteria (#9 and #11, respectively). This amendment also clarified that clinical management should be considered in the case of ALT level increments of at least 2 × baseline and >ULN and AST level increments of at least 2 × ULN.

Amendment 2 also specified that the lead-in phase would occur for a minimum of 26 weeks, ending at or before Visit D. It was clarified that the wash out period would be 3 days for regular-acting FIX products and 10 days for extended half-life FIX products. The amendment specified that study visits should be scheduled on the day routine prophylactic FIX replacement treatment was planned to be administered to ensure FIX activity was at its trough. Post-treatment follow-up assessments could be conducted at home, as appropriate. Alpha-fetoprotein and FIX gene sequence analyses were added in this amendment. It was clarified that the short form of the iPAQ would be used; the long form of the iPAQ was completed by some subjects prior to this amendment. For the optional MSKUS sub-study, it was clarified that if it was not possible to obtain the MSKUS at screening, obtaining this first MSKUS at a later timepoint was allowed.

In Amendment 3, an abdominal ultrasound to screen for liver malignancy was added. Exclusion criterion #3 was updated to allow subjects with total bilirubin >2 × ULN if this elevation was caused by Gilbert disease. It was specified that subjects could have received their dose of continuous routine FIX on the day of AMT-061 dosing; blood sampling was to occur prior to FIX administration and the requirement that this occurred at the clinic was removed. It was specified that the time period between Visit LX and Visit L-Final could be less than 2 months as long as the total lead-in period was a minimum of 26 weeks. It was also clarified that if subjects were not able to enter details of their FIX replacement therapy or bleeding episodes into the e-diary, the site could have entered the information into the eCRF if the subject provided sufficient source documentation. Additionally, guidelines for use of FIX for subjects undergoing major surgery were added.

In Amendment 4, the primary objective and endpoints were updated to include FIX activity after 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 FIX prophylaxis during the lead-in phase,

which were both previously secondary objectives/endpoints. This amendment also clarified that paper diaries were to be used during the long-term follow-up phase to document bleeding episodes and FIX use.

In Amendment 5, the frequency of abdominal ultrasounds in the long-term follow-up phase was increased from yearly to every 6 months, and it was specified that abdominal ultrasounds could be performed at an unscheduled visit during the long-term follow-up, if judged relevant by the Investigator. It was clarified that subjects on continuous routine FIX prophylaxis during the long-term follow-up phase were required to contact the site staff immediately in case of a bleeding episode and/or FIX use different from their routine FIX prophylaxis, in addition to completing the paper diaries. Local laboratory assessments of FIX, ALT, and AST were added to the long-term follow-up. Additionally, possible options for causality assessments for AEs (related, probably related, possibly related, and not related) were updated to align with what was collected on the eCRFs.

In Amendment 6, study objectives and endpoints were updated, based on input from the FDA. The Hemgenix, was updated to focus on ABR, 52 weeks following establishment of stable FIX expression (Months 6 to 18 post-treatment). The previous primary objectives and endpoints related to endogenous FIX activity at 6 months and 12 months were moved to be the first and second secondary efficacy endpoints, respectively, and endogenous FIX 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 titres using the luciferase based nAb assay was added as a secondary efficacy endpoint (as a "correlation" analysis).

For the 6-month analyses, a less refined contamination rule was used, whereby the date of exogenous FIX infusion and the subsequent 9 days (10 discrete calendar days in total) were considered to be days of contamination with FIX. The 12-month and 18-month analyses used the more refined definition of contamination, based on 5 half-lives. 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. To align with the updated endpoints, the statistical analyses were also reordered and timing clarified. 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 updated, although the sample size remained at 50 subjects. Details on the interim and final analyses were updated. Clarification was added that during the long-term follow up, quarterly contact (±2 weeks) should occur to monitor for AEs, proper completion of study-specific paper diaries, and proper reporting of FIX usage and bleeding episodes. 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.

Protocol Deviations

The majority of protocol deviations were related to timing of study visits, questionnaire completion, or absence or incorrect performance of laboratory tests. The impact on the efficacy outcomes is considered to be minimal.

- **Baseline data**

Table 2. Summary of Demographic and Baseline Characteristics (Safety Population)

Characteristic	Lead-in Safety Population Incl. Lead-in Discontinuers (N = 67)	Post-treatment Safety Population/FAS (N = 54)	PP Population (N = 53)
Age (years), n[1]	67	54	53
Mean (SD)	42.8 (16.2)	41.5 (15.8)	40.9 (15.5)
Median (Q1-Q3)	38.0 (31.0-55.0)	37.0 (30.0-53.0)	37.0 (30.0-50.0)
Sex, n (%)			
Male	67 (100.0)	54 (100.0)	53 (100.0)
Race, n (%)			
White	50 (74.6)	40 (74.1)	40 (75.5)
Other	7 (10.4)	6 (11.1)	5 (9.4)
Missing	5 (7.5)	5 (9.3)	5 (9.4)
Asian	3 (4.5)	2 (3.7)	2 (3.8)
Black or African American	2 (3.0)	1 (1.9)	1 (1.9)
Ethnicity, n (%)			
Non-Hispanic or Latino	56 (83.6)	45 (83.3)	44 (83.0)
Hispanic or Latino	6 (9.0)	4 (7.4)	4 (7.5)
Missing	5 (7.5)	5 (9.3)	5 (9.4)
Height (cm), n	66	54	53
Mean (SD)	176.9 (7.9)	176.5 (8.2)	176.8 (8.0)
Median (Q1-Q3)	176.5 (172.0-182.0)	176.5 (172.0-182.0)	177.0 (172.0-182.0)
Min, Max	153, 197	153, 197	153, 197
Weight (kg), n	66	54	53
Mean (SD)	87.2 (20.0)	85.1 (19.3)	85.5 (19.3)
Median (Q1-Q3)	85.5 (74.0-96.0)	84.0 (74.0-93.0)	84.0 (75.0-93.0)
Min, Max	58, 169	58, 169	58, 169
BMI (kg/m^2), n	66	54	53
Mean (SD)	27.7 (5.4)	27.2 (5.1)	27.2 (5.1)
Median (Q1-Q3)	26.7 (23.8-30.1)	26.2 (23.8-29.1)	26.3 (23.8-29.1)
Min, Max	21, 51	21, 51	21, 51

Abbreviations: BMI = body mass index; FAS = Full Analysis Set; Incl. = including; Max = maximum; Min = minimum; PP = Per-Protocol; Q = quartile, SD = standard deviation.
[1.] Age was the age at the time of Informed Consent.

21/54 (38.9%) subjects had anti-AAV5 nAbs before dosing with a median titre of 1:56.9 (range: 1:9 to 1:3212).

Table 3. Summary of Medical History Relating to Haemophilia B (Safety Population)

Characteristic	Lead-in Safety Population Incl. Lead-in Discontinuers (N = 67)	Post-treatment Safety Population/FAS (N = 54)	PP (N = 53)
Bleeding Episodes in Year Prior to Screening, n (%) [# of Episodes]			
Any Bleeding Episodes	53 (79.1) [258]	44 (81.5) [215]	43 (81.1) [214]
Joint Bleeding Episodes	33 (49.3) [155]	30 (55.6) [132]	29 (54.7) [131]
Spontaneous Bleeding Episodes	36 (53.7) [141]	32 (59.3) [118]	31 (58.5) [117]
Traumatic Bleeding Episodes	26 (38.8) [72]	20 (37.0) [64]	20 (37.7) [64]
Unknown	14 (20.9) [45]	11 (20.4) [33]	11 (20.8) [33]
Bleeding Episodes in Year Prior to Screening, n (%)			
0 Bleeding Episodes	14 (20.9)	10 (18.5)	10 (18.9)
1 Bleeding Episodes	11 (16.4)	9 (16.7)	8 (15.1)
2 Bleeding Episodes	14 (20.9)	10 (18.5)	10 (18.9)
3 Bleeding Episodes	8 (11.9)	8 (14.8)	8 (15.1)
4 Bleeding Episodes	4 (6.0)	4 (7.4)	4 (7.5)
5 Bleeding Episodes	2 (3.0)	2 (3.7)	2 (3.8)
6 Bleeding Episodes	2 (3.0)	2 (3.7)	2 (3.8)
7 Bleeding Episodes	2 (3.0)	2 (3.7)	2 (3.8)
8 Bleeding Episodes	3 (4.5)	2 (3.7)	2 (3.8)
10 Bleeding Episodes	1 (1.5)	0	0
11-15 Bleeding Episodes	4 (6.0)	3 (5.6)	3 (5.7)
>20 Bleeding Episodes	2 (3.0)	2 (3.7)	2 (3.8)
FIX Replacement Therapy Type, n (%)			
Prophylactic	67 (100.0)	54 (100.0)	53 (100.0)
On-demand	5 (7.5)	4 (7.4)	4 (7.5)
Most Recent Pre-Screening FIX Therapy Category, n (%)			
Extended Half-life	40 (59.7)	31 (57.4)	30 (56.6)
Standard Half-Life	27 (40.3)	23 (42.6)	23 (43.4)
HIV Status, n (%)			
Negative	63 (94.0)	51 (94.4)	50 (94.3)
Positive	4 (6.0)	3 (5.6)	3 (5.7)
Hepatitis B Infection, n (%)			
Prior Resolved[4]	13 (19.4)	9 (16.7)	9 (17.0)
Hepatitis C Infection, n (%)			

Prior or Ongoing[4]	38 (56.7)	31 (57.4)	30 (56.6)
Prior Resolved	34 (50.7)	28 (51.9)	27 (50.9)
Ongoing	4 (6.0)	3 (5.6)	3 (5.7)
Positive at Screening[5]	1 (1.5)	1 (1.9)	1 (1.9)

Abbreviations: FAS = Full Analysis Set; FIX = Factor IX; HIV = human immunodeficiency virus; Incl. = including; PP = Per Protocol; SD = standard deviation.

1. Duration was calculated based on the date the subject was initially diagnosed with hemophilia B according to the Case Report Form.
2. FIX plasma level <1%.
3. FIX plasma level ≥1% and ≤2%.
4. Prior or ongoing per reported medical history. All subjects tested negative pre-dose.
5. Subjects positive at screening had "Hepatitis C Virus RNA = Detected" for Hepatitis C. Subject was positive at screening and negative at L-Final visit

- **Numbers analysed**

Table 4. Data Sets Analysed CT-AMT-061-02

	Total (N=75)
	N (%)
Safety Population[1]	67/75 (89.3)
Lead-in Safety Population[2]	67/75 (89.3)
Lead-in Discontinuers (i.e., Not Treated with AMT-061)[3]	13/67 (19.4)
Post-treatment Safety Population (i.e., Treated with AMT-061)[4]	54/67 (80.6)
Full Analysis Set[5]	54/54 (100.0)
Per-Protocol Population[6]	53/54 (98.1)
Patient Reported Outcomes, Burdens, and Experiences (PROBE) Sub-study[7]	49/54 (90.7)

1. The Safety Population included subjects in either the Lead-in Safety Population or the Post-treatment Safety Population.
2. The Lead-in Safety Population included subjects who received lead-in treatment (i.e., who were enrolled into the lead-in period).
3. The Lead-in Discontinuers Population included subjects who entered the lead-in period but discontinued from the study prior to AMT-061 dosing.
4. The Post-treatment Safety Population included subjects who received AMT-061, irrespective of any protocol deviations.
5. The Full Analysis Set included subjects who enrolled, entered the lead-in period, were dosed with AMT-061, and provided at least one efficacy endpoint assessment for any efficacy endpoint subsequent to AMT-061 dosing.
6. The Per-Protocol Population included all subjects from the Full Analysis Set who adhered to a stable and adequate prophylaxis use during the lead-in period, completed at least 18 months of efficacy assessments (52 weeks after achieving stable Factor IX expression), and had no major protocol deviations that impacted the interpretation of efficacy (as documented at the data review meeting).
7. The PROBE sub-study was the subset of the Full Analysis Set that had at least one post-treatment assessment of the given assessment tool.

- **Outcomes and estimation**

Primary Endpoint

Table 5. Summary of Bleeding Episodes and Annualised Bleeding Rates (Full Analysis Set)

	All Bleeding Episodes			FIX-treated Bleeding Episodes			All Bleeding Episodes for Subjects with anti-AAV5 NAb <3000		
	≥6-month Lead- in Period (N = 54)	Month 7-18 (N = 54)	Month 7-24 (N = 54)	≥6-month Lead- in Period (N = 54)	Month 7-18 (N = 54)	Month 7-24 (N = 54	≥6-month Lead- in Period (N = 53)	Month 7-18 (N = 53)	Month 7-24 (N = 53)
Number of Subjects With a Bleeding Episode n (%)	40 (74.1)	20 (37.0)	27 (50.0)	37 (68.5)	15 (27.8)	19 (35.2)	40 (75.5)	19 (35.8)	26 (49.1)
Number of Subjects with Zero Bleeding Episodes, n (%)	14 (25.9)	34 (63.0)	27 (50.0)				13 (24.5)	34 (64.2)	27 (50.9)
Cumulative Number of Bleeding Episodes, n	136	54	74	118	30	43	136	49	69
Cumulative Number of Person-years Observed for Bleeding Episodes, n	33.12	49.78	74.56	33.12	49.78	74.56	32.60	49.77	74.56
Unadjusted ABR[1]	4.11	1.08	0.99	3.56	0.60	0.58	4.17	0.98	0.93
Adjusted ABR (95% CI)[2]	4.19 (3.22, 5.45)	1.51 (0.81, 2.82)	1.51 (0.83, 4.76)	3.65 (2.82, 4.74)	0.84 (0.41, 1.73)	0.99 (0.48, 2.03)	3.89 (2.93, 5.16)	1.07 (0.63, 1.82)	1.09 (0.67, 1.79)
Rate Ratio (Post-treatment/Lead-in)[2]		0.36	0.36		0.23	0.27		0.28	0.28
Two-sided 95% Wald CI[3]		0.20, 0.64	0.21, 0.63		0.12, 0.46	0.14, 0.54		0.17, 0.43	0.17, 0.46
p-value[4]		0.0002	0.0002		<0.0001	0.0001		<0.0001	<0.0001

Abbreviations: ABR = annualised bleeding rate; CI = confidence interval; FIX = Factor IX; NAb = neutralizing antibody.
Person-time during the post-treatment period (on any day that began) within 5 half-lives subsequent to exogenous FIX use at risk of (having) a bleeding episode. Nevertheless, bleeding episodes during such person-time were still counted.

1. Unadjusted ABR was calculated as the ratio of the number of bleeding episodes to the time of observation (in years).
2. Adjusted ABR and comparison of ABR between the lead-in and post-treatment period was estimated from a repeated measures generalised estimating equations negative binomial regression model accounting for the paired design of the trial with an offset parameter to account for the differential collection periods. Treatment period was included as a categorical covariate.
3. The upper limit of the confidence interval of the rate ratio was compared to the non-inferiority margin of 1.8. If the upper limit was less than 1.8, then non-inferiority was declared.
4. One-sided p-value ≤0.025 for post-treatment/lead-in <1 was regarded as statistically significant. For Month 7-24, p-values not adjusted for multiplicity.

The adjusted ABR for all bleeding episodes was reduced following AMT-061 treatment and stable FIX expression, from a rate of 4.19 (95% CI: 3.22, 5.45) for the ≥6-month lead-in period to 1.51 (95%

CI:0.81, 2.82) for Months 7 to 18 of the post-treatment period (64% reduction [95% CI: 36%, 80%; period was 0.36 (95% Wald CI: 0.20, 0.64). As the upper limit of the Wald CI was less than 1.8, non-inferiority can be declared vs. the lead-in standard of care FIX prophylaxis.

During the ≥6-month lead-in period (cumulative 33.12 person-years of observation), the majority of subjects who later received treatment (40/54 [74.1%]) experienced bleeding episodes.

A total of 136 bleeding episodes were reported for the lead-in period, including 118 FIX-treated bleeding episodes. The majority of bleeding episodes (118/136) were very mild to moderate in severity; 14 severe and 4 very severe bleeding episodes were reported in 10/54 (18.5%) subjects and 3/54 (5.6%) subjects, respectively. Traumatic and spontaneous bleeding episodes were reported in 29/54 (53.7%) and 24/54 (44.4%) subjects, respectively. The most common locations of bleeding episodes in the lead-in period were joints (59.3%) and muscle (31.5%).

During Months 7 to 18 of the post-treatment period, following AMT-061 treatment and stable FIX expression (cumulative 49.78 person-years observed), the majority of treated subjects (34/54 [63.0%]) had zero bleeding episodes; bleeding episodes were reported in 20/54 (37.0%) subjects.

During Months 7 to 18 of the post-treatment period, 54 bleeding episodes were reported including 30 FIX-treated bleeding episodes. The majority of bleeding episodes (43/54) were very mild to moderate in severity; 7 severe and 2 very severe bleeding episodes were reported in 7/54 (13.0%) of subjects and 2/54 (3.7%) subjects, respectively and severity was missing for 2 episodes. Traumatic and spontaneous bleeding episodes were reported in 12/54 (22.2%) and 9/54 (16.7%) subjects, respectively. The most common locations of bleeding episodes during this post-treatment period were joint (20.4) and surface (14.8%).

Sensitivity analyses demonstrated the robustness of the ABR results. For FIX-treated bleeding episodes, the adjusted ABR was 3.65 (95% CI: 2.82, 4.74) for the ≥6-month lead-in period and 0.84 (95% CI: 0.41, 1.73) for Months 7 to 18 of the post-treatment period (77% reduction [95% CI: 54%, 88%, p <0.0001), with an observed ABR rate ratio for the Month 7 to 18 post-treatment period to lead-in period of 0.23 (95% Wald CI: 0.12, 0.46). Similar ABR results were observed for the Months 7 to 18 post-treatment period when the analysis was conducted with the PP Population, irrespective of FIX use during the post-treatment period, including only new and true exogenous bleeding episodes, including only new and true exogenous FIX-treated bleeding episodes, and excluding person-time with contamination from systemic corticosteroids.

Additionally, in the 53/54 subjects with baseline (i.e. pre-dose) anti-AAV5 nAb titre <3000, the mean adjusted ABR was 1.07 during Months 7 to 18, with an observed rate ratio of 0.28 (95% Wald CI: 0.17, 0.43).

ABR was significantly reduced during Months 7 to 18 after AMT-061 treatment compared to the lead-in period for most of the subgroups analysed, with rate ratios (post-treatment/lead-in) ranging from 0.16 to 0.57 (p <0.025 for most subgroups (not adjusted for multiplicity). Exceptions to this included subjects with a positive anti-AAV5 nAb titre at baseline (N = 21; rate ratio = 1.77), non-White subjects (N = 14; rate ratio = 9.14), and subjects who had target joints at screening (N = 10; rate ratio = 13.42); however, for these subgroups, the unadjusted ABR at Months 7 to 18 post-treatment was less than that for the lead-in period. In the ABR analysis, all bleeding episodes were counted but person-time during the post-treatment period (on any day that began) within 5 half-lives subsequent to exogenous FIX use was not considered to be time at risk of (having) a bleeding episode, which may have led to higher subject estimates for some subgroups.

The higher ABR rate ratio in the baseline anti-AAV5 nAb-positive subgroup was driven by a single subject with a pre-dose anti-AAV5 nAb titre of 3212.3. This subject did not respond to treatment with AMT-061 and was on prophylactic treatment, receiving 30 FIX injections during Months 7 to 18. Time

within 5 half-lives of a FIX injection was removed from the time at risk, which resulted in approximately one day (1.09 days) at risk during Months 7 to 12. During this time, this subject had 4 spontaneous and 1 unknown bleeds, resulting in an ABR of 1673.97. When this subject was excluded from the analysis, superiority was reached for the baseline anti-AAV5 nAb-positive subgroup with an adjusted rate ratio of 0.30 (95% CI 0.15, 0.62).

Secondary Endpoints

FIX Activity at 6 Months, 12 Months, and 18 Months Post-AMT-061 Administration

Table 6. FIX Activity from One-stage (aPTT-based) Assay at 6 Months, 12 Months, and 18 Months Post-AMT-061 Administration (Full Analysis Set)

	Result			Change from Baseline		
Visit[1]	n	Mean (SD)	Median (Min, Max)	LS Mean (SE)[2]	95% CI	p-value[3]
Baseline	54	1.19 (0.39)	1.00 (1.0, 2.0)			
Month 6	51	38.95 (18.72)	37.30 (8.2, 97.1)	36.18 (2.432)	31.41, 40.95	<0.0001
Month 12	50	41.48 (21.71)	39.90 (5.9, 113.0)	38.81 (2.442)	34.01, 43.60	<0.0001
Month 18	50	36.90 (21.40)	33.55 (4.5, 122.9)	34.31 (2.444)	29.52, 39.11	<0.0001
Month 24	50	36.66 (18.96)	33.85 (4.7, 99.2)	34.13 (2.325)	29.57, 38.69	<0.0001

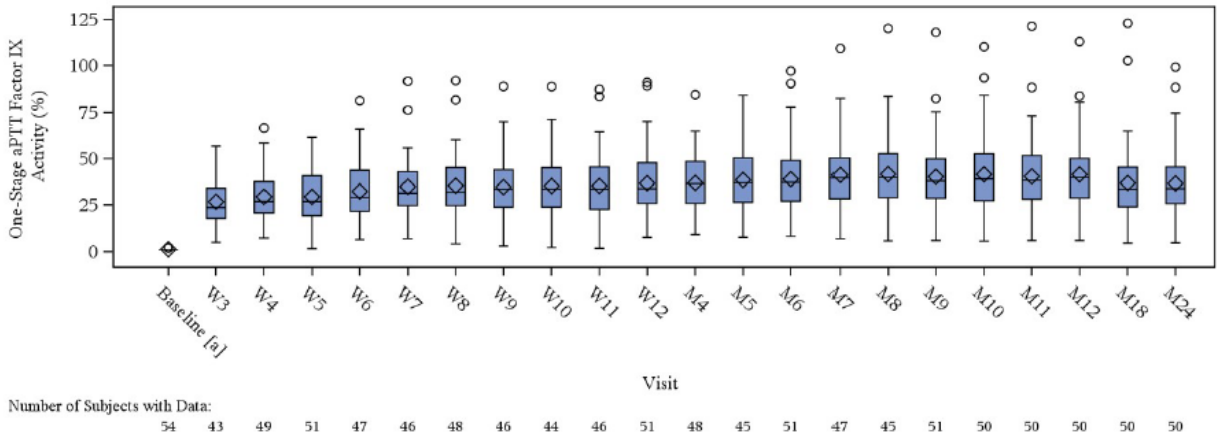
Abbreviations: aPTT = activated Partial Thromboplastin Time; CI = confidence interval; FIX = Factor IX; LS = least squares; Max = Maximum; Min = Minimum; SD = standard deviation; SE = standard error.

1. "Uncontaminated" meant that the blood sampling did not occur within 5 half-lives of exogenous FIX use. Both the date and time of exogenous FIX use and blood sampling were considered in determining contamination. FIX levels beginning with the Week 3 assessment were used in the analysis. Subjects with zero uncontaminated central- laboratory post-AMT-061 values had their change from baseline assigned to zero for this analysis, and had their post- baseline values set equal to their baseline value; however, the ratio of chromogenic to one-stage was not imputed. Baseline FIX was imputed based on subject's historical hemophilia B severity documented on the case report form. If the subject had documented severe FIX deficiency (FIX plasma level <1%), their baseline FIX activity level was imputed as 1%. If the subject had documented moderately severe FIX deficiency (FIX plasma level ≥1% and ≤2%,) their baseline FIX activity level was imputed as 2%.

2. LS mean from repeated measures linear mixed model with visit as a categorical covariate.

3. One-sided p-value ≤0.025 for post-treatment >baseline was regarded as statistically significant. For Month 24, p-value not adjusted for multiplicity.

Figure 3. Box Plot of Uncontaminated Central Laboratory One-stage (aPTT-based) FIX Activity (%) Over Time During the Post-Treatment Period (Full Analysis Set)



Abbreviations: aPTT = activated partial thromboplastin time; FIX = Factor IX; M = month; SE = standard error; W = week.
"Uncontaminated" meant that the blood sampling did not occur within 5 half-lives of exogenous FIX use. FIX levels beginning with the Week 3 assessment were used in the analysis. Both the date and time of the exogenous FIX use (start) and the blood sampling were considered in determining contamination. Subjects with zero uncontaminated central laboratory post-AMT-061 values had their post-baseline values set equal to their baseline value.
The lower and upper edges of the box correspond to the interquartile range, the 25th, and 75th percentile. The line at the middle of the box corresponds to the median. The whiskers (horizontal lines connected to vertical lines) show the lowest and highest observation within 1.5 times the interquartile range of the bottom and top of the box, respectively. The diamond is the arithmetic mean. Any points outside of the whiskers are plotted individually.
[a] Baseline FIX was imputed based on subject's historical hemophilia B severity documented on the case report form. If the subject had documented severe FIX deficiency (FIX plasma level <1%), their baseline FIX activity level was imputed as 1%. If the subject had documented moderately severe FIX deficiency (FIX plasma level ≥1% and ≤2%), their baseline FIX activity level was imputed as 2%. The SE was not provided at baseline.

FIX activity levels showed clinically relevant values at month 6, continued to increase until month 12 and then declined slightly until month 18 and were steady at month 24. No subject recorded values >150%.

Sensitivity analyses demonstrated the robustness of these results. A similar change from baseline in FIX activity was observed when the analysis was conducted in the PP Population and when missing FIX levels were imputed subjects with 0, uncontaminated values were excluded, values contaminated by systemic corticosteroid exposure were excluded, and when the alternative FIX contamination rule was used. Similar results were observed in subjects with baseline anti-AAV5 nAb titre <3000.

FIX activity levels measured by one-stage and chromogenic assay showed a similar discrepancy as in trial CT-AMT-061-01, with the chromogenic assay returning significantly lower levels of FIX activity.

Annualised Consumption of FIX Replacement Therapy

Table 7: Annualised Consumption of FIX Replacement Therapy (IU/year; Full Analysis Set)

	⩾6-month Lead-in Period (N = 54)	Post-treatment Period			
		Month 0-6 (N = 54)	Month 7-12 (N = 54)	Month 13-18 (N = 54)	Month 19-24 (N = 53)[1]
Annualised Exogenous FIX Consumption (IU/year), n	54	54	54	54	53
Unadjusted Mean	257,338.8	12,912.9	8399.1	8486.6	9750.8
(SD)	(149,013.1)	(37,093.1)	(29,720.9)	(28,770.2)	(29,140.4)
Min; Max	83,541; 755,892	0; 204,899	0; 156,536	0; 180,618	0; 155,680

Abbreviations: FIX = Factor IX; IU = international units; Max = maximum; Min = minimum; SD = standard deviation.
FIX replacement therapy use for invasive procedures was not included in the analysis.
Lead-in period time was the total number of days during which the subject was in the lead-in period divided by 365.25. Post-treatment period time was the number of days of observation within the time interval, excluding information prior to Day 21.
[1] One subject died prior to Month 19.

Table 8. Annualised Use of FIX Replacement Therapy (Infusions/year; Full Analysis Set)

	≥6-month Lead-in Period (N = 54)	Post-treatment Period			
		Month 0-6 (N = 54)	Month 7-12 (N = 54)	Month 13-18 (N = 54)	Month 19-24 (N = 53)
Number of Subjects Using FIX Replacement Therapy, n (%)	54 (100.0)	14 (25.9)	10 (18.5)	11 (20.4)	13 (24.5)
Number of Infusions of FIX Replacement Therapy, n	2380	85	70	64	42
Mean (per subject)	44.1	1.6	1.3	1.2	0.8
Median (Min, Max; per subject)	37.0 (12, 107)	0.0 (0, 34)	0.0 (0, 39)	0.0 (0, 26)	0.0 (0, 13)
Number of Person-years Observed for FIX Usage	33.12	24.10	26.91	26.12	25.85
	≥6-month Lead-in Period (N = 54)	Post-treatment Period			
		Month 0-6 (N = 54)	Month 7-18 (N = 54)	Month 7-24 (N=54)	Year 0-1 (N = 54)
Cumulative Number of Infusions of FIX Therapy	2380	85	134	176	155

Cumulative Number of Person-years Observed for FIX Usage	33.12	24.10	53.03	79.18	51.01
Unadjusted Annualised Infusion Rate[1]	71.87	3.53	2.53	2.22	3.04
Adjusted Annualised Infusion Rate, n Adjusted Rate	72.49		2.53	2.54	3.04
(95% CI)[2]	(63.52, 82.71)		(0.92, 6.96)	(0.98, 6.59)	(1.14, 8.12)
Rate Ratio (Post-treatments/ Lead-in)[2]			0.03	0.04	0.04
Two-sided 95% Wald CI[3]			0.01, 0.10	(0.01, 0.09)	0.02, 0.11
p-value[4]			<0.0001	<0.0001	<0.0001

Abbreviations: CI = confidence interval; FIX = Factor IX; Max = maximum; Min = mi

Post-treatment period time was the number of days of observation within the time interval, excluding information prior to Day 21.

[1.] Unadjusted infusion rate was calculated as the ratio of the number of infusions of FIX to the time of observation (in years). Usage related to invasive procedures was not included.

[2.] Adjusted infusion rate and comparison of infusion rate between lead-in and post-treatment period was estimated from a repeated measures generalized estimating equations negative binomial regression model accounting for the paired design of the trial with an offset parameter to account for the differential collection periods. Treatment period was included as a categorical covariate.

[3.] One-sided p-value ≤0.025 for post-treatment/lead-in <1 was regarded as statistically significant. For Month 7-18, p-value adjusted for multiplicity.

Proportion of Subjects Remaining Free of Previous Continuous Routine Prophylaxis

Following treatment with AMT-061, 52/54 (96.3%) subjects discontinued FIX prophylaxis and remained free of routine FIX prophylaxis from Day 21 through to Months 7 to 24.

The other 2 subjects included a subject who received a partial dose of AMT-061 and a subject who had a high anti-AAV5 nAb titre at pre-dose (titre = 3212.3).

Percentage of Subjects with Trough FIX Activity <12% of Normal

The percentage of subjects attaining FIX activity <12% of normal (measured by the one-stage [aPTT-based] assay) was compared between the lead-in period and post-treatment period; FIX activity levels within 5 half-lives of exogenous FIX use were not included in the analysis.

By the end of the ≥6-month lead-in period, 43/54 (79.6%) subjects had FIX activity <12% of normal. Three months following treatment with AMT-061, FIX activity was <12% of normal in 4/51 (7.8%) subjects. This improvement in FIX activity was sustained through Month 12 of the post-treatment period, with 4/50 (8.0%) subjects having FIX activity <12% of normal. At Month 18 and Month 24, there were 3/50 (6.0%) subjects and 5/50 (10.0%) subjects with FIX activity <12% of normal, respectively.

Annualised Bleeding Rates: Superiority Assessment

The ABR for all bleeding episodes during Months 7 to 18 was reduced by 64% following AMT-061 treatment, with an observed ABR rate ratio for Months 7 to 18 of the post-treatment period to ≥6-

month lead-in period of 0.36 (95% Wald CI: 0.20, 0.64) and a one-sided p-value of 0.0002, demonstrating superiority of AMT-061 compared to standard of care routine FIX prophylaxis.

Superiority of AMT-061 compared to standard of care routine FIX prophylaxis was also demonstrated with FIX-treated bleeding episodes. The ABR for FIX-treated bleeding episodes during Months 7 to 18 was reduced by 77% following AMT-061 treatment, with an ABR for Months 7 to 18 of the post-treatment period of 0.84 (95% CI: 0.41, 1.73) and a rate ratio (compared to lead-in) of 0.23 (Wald 95% CI: 0.12, 0.46; p <0.0001).

At Month 24, an analysis for ABR for all bleeding episodes and for FIX-treated episodes could show superiority over standard of care, but adjustment for multiplicity was not done for these results.

Rate of Spontaneous Bleeding Episodes

Table 9. Summary of Spontaneous Bleeding Episodes (Full Analysis Set)

	Spontaneous Bleeding Episodes			FIX-treated Spontaneous Bleeding Episodes		
	≥6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)	Month 7-24 (N = 54)	≥6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)	Month 7-24 (N = 54)
Number of Subjects With a Bleeding Episode, n (%)	24 (44.4)	9 (16.7)	11 (20.4)	22 (40.7)	6 (11.1)	8 (14.8)
Cumulative Number of Bleeding Episodes, n	50	14	18	44	11	15
Cumulative Number of Person-years Observed, n	33.12	49.78	74.56	33.12	49.78	74.56
Unadjusted ABR[1]	1.51	0.28	0.24	1.33	0.22	0.20
Adjusted ABR (95% CI)[2]	1.52 (1.01, 2.30)	0.44 (0.17, 1.12)	0.38 (0.16, 0.89),	1.34 (0.87, 2.06)	0.45 (0.15, 1.39)	0.42 (0.15, 1.19)
Rate Ratio (Post-treatment/ Lead-in)[2]		0.29	0.25		0.34	0.31
Two-sided 95% Wald CI[2]		0.12, 0.71	0.11, 0.57		0.11, 1.00	0.11, 0.87
p-value[3]		0.0034	0.0005		0.0254	0.0127

Abbreviations: ABR = annualized bleeding rate; CI = confidence interval; FIX = Factor IX.
Person-time during the post-treatment period (on any day that began) within 5 half-lives subsequent to exogenous FIX use was not considered to be time at risk of (having) a bleeding episode. Nevertheless, bleeding episodes during such person-time were still counted.

1. Unadjusted ABR was calculated as the ratio of the number of bleeding episodes to the time of observation (in years).
2. Adjusted ABR and comparison of ABR between the lead-in and post-treatment period was estimated from a repeated measures generalized estimating equations negative binomial regression model accounting for the paired design of the trial with an offset parameter to account for the differential collection periods. Treatment period was included as a categorical covariate. Lead-in period data for the Month 7 to 24 comparisons not shown in table; these data are available in the outputs listed below as Source for Month 7 to 24.
3. One-sided p-value ≤0.025 for post-treatment/lead-in <1 was regarded as statistically significant. For Month 7-24, p-values not adjusted for multiplicity.

Spontaneous bleeding episodes were only experienced by 16.7% of subjects at month 18 and 20.4% of subjects at month 24 post-treatment, while 44.4% of subjects reported such events during the lead-in. This is considered a clinically relevant improvement over FIX-prophylaxis, because many spontaneous bleeds occur in joints and joint health continues to deteriorate over time despite prophylaxis.

Rate of Joint Bleeding Episodes

Table 10. Summary of Joint Bleeding Episodes (Full Analysis Set)

	Joint Bleeding Episodes			FIX-treated Joint Bleeding Episodes		
	≥6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)	Month 7-24 (N = 54)	≥6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)	Month 7-24 (N = 54)
Number of Subjects With a Bleeding Episode, n (%)	32 (59.3)	11 (20.4)	15 (27.8)	31 (57.4)	9 (16.7)	12 (22.2)
Cumulative Number of Bleeding Episodes, n	77	19	26	70	16	22
Cumulative Number of Person-years Observed, n	33.12	49.78	74.56	33.12	49.78	74.56
Unadjusted ABR[1]	2.33	0.38	0.35	2.11	0.32	0.30
Adjusted ABR (95% CI)[2]	2.35 (1.74, 3.16)	0.51 (0.23, 1.12)	0.46 (0.24, 0.89)	2.13 (1.58, 2.88)	0.44 (0.19, 1.00)	0.40 (0.20, 0.83)
Rate Ratio (Post-treatment/ Lead-in)[2]		0.22	0.20		0.20	0.19
Two-sided 95% Wald CI[2]		0.10, 0.46	0.10, 0.37		0.09, 0.45	0.09, 0.38
p-value[3]		<0.0001	<0.0001		<0.0001	<0.0001

Abbreviations: ABR = annualized bleeding rate; CI = confidence interval; FIX = Factor IX.
Person-time during the post-treatment period (on any day that began) within 5 half-lives subsequent to exogenous FIX use was not considered to be time at risk of (having) a bleeding episode. Nevertheless, bleeding episodes during such person-time were still counted.

1. Unadjusted ABR was calculated as the ratio of the number of bleeding episodes to the time of observation (in years).
2. Adjusted ABR and comparison of ABR between the lead-in and post-treatment period was estimated from a repeated measures generalized estimating equations negative binomial regression model accounting for the paired design of the trial with an offset parameter to account for the differential collection periods. Treatment period was included as a categorical covariate. Lead-in period data for the Month 7 to 24 comparisons not shown in table; these are available in the outputs listed below as Source for Month 7 to 24.
3. One-sided p-value≤0.025 for post-treatment/lead-in <1 was regarded as statistically significant. For Month 7-24, p-values not adjusted for multiplicity.

20.4% of subjects reported joint bleeding episodes post-treatment until month 18, and 27.8% reported joint bleeds until month 24, while 59.3% of subjects reported such events during the lead-in, which is a clear improvement and represents a clinically meaningful benefit.

FIX Activity Levels and Anti-AAV5 Neutralizing Antibodies

Overall, 21 subjects had pre-existing nAbs against AAV5 at baseline (i.e., pre-dose), prior to AMT-061 treatment. Positivity for the presence of anti-AAV5 nAbs required a titre ≥LOD of 7. At baseline, anti-AAV5 nAb titres were between LOD and <3000 (range: 8.5 to 678.2) for 20/54 (37.0%) subjects treated with AMT-061, and was 3212.3 for 1 subject (Subject 15-42-259). Sensitivity analyses conducted within the FAS population for subjects with baseline anti-AAV5 nAb titre <3000 did not include data for this subject.

Baseline mean (SD) FIX activity was similar between subjects with pre-existing anti-AAV5 nAbs (1.24 [0.44]%) and those without pre-existing anti-AAV5 nAbs (1.15 [0.36]%; Table 17). At Month 6 post-treatment with AMT-061, FIX activity was 35.91% and 40.61% for subjects with and without pre-existing anti-AAV5 nAbs, respectively, and was significantly increased from baseline with LS mean increases of 30.79% (95% CI: 23.26, 38.32; p <0.0001) and 39.46% (95% CI:33.23, 45.69; p <0.0001), respectively. At Month 12 post-treatment, FIX activity was 35.54% and 44.82% of normal for subjects with and without pre-existing anti-AAV5 nAbs. At 18 months post-treatment, FIX activity was 31.14% and 39.87% for subjects with and without pre-existing anti-AAV5 nAbs, respectively, with LS mean increases from baseline of 26.83% (95% CI: 19.24, 34.41; p <0.0001) and 38.72% (95% CI: 32.49, 44.95; p <0.0001), respectively. At 24 months post-treatment, mean FIX activity was 32.98% and 38.55% for subjects with and without pre-existing anti-AAV5 NAbs, respectively, with LS mean increases from baseline of 28.35% (95% CI: 20.62, 36.08; p <0.0001) and 37.40% (95% CI: 31.64, 43.16; p <0.0001), respectively.

Table 11. Summary of FIX Activity (%) From One-stage (aPTT-based) Assay in the Post-Treatment Period for Subjects With and Without Pre-Existing Neutralizing Antibodies to AAV5 (Full Analysis Set)

Visit	Result			Change from Baseline		
	n	Mean (SD)	Median (Min, Max)	LS Mean (SE)[1]	95% CI	p-value[2]
Pre-existing anti-AAV5 NAbs						
Baseline[3]	21	1.24 (0.44)	1.00 (1.0, 2.0)			
Post-treatment Month 6	18	35.91 (19.02)	35.60 (8.2, 90.4)	30.79 (3.827)	23.26, 38.32	<0.0001
Post-treatment Month 12	18	35.54 (17.84)	39.95 (8.5, 73.6)	31.59 (3.847)	24.02, 39.16	<0.0001
Post-treatment Month 18	17	31.14 (13.75)	32.00 (10.3, 57.9)	26.83 (3.854)	19.24, 34.41	<0.0001
Post-treatment Month 24	17	32.98 (18.51)	33.50 (9.1, 88.3)	28.35 (3.928)	20.62, 36.08	<0.0001

Visit	Result			Change from Baseline		
	n	Mean (SD)	Median (Min, Max)	LS Mean (SE)[1]	95% CI	p-value[2]
Without Pre-existing anti-AAV5 NAbs						
Baseline[3]	33	1.15 (0.36)	1.00 (1.0, 2.0)			
Post-treatment Month 6	33	40.61 (18.64)	37.30 (8.4, 97.1)	39.46 (3.172)	33.23, 45.69	<0.0001
Post-treatment Month 12	32	44.82 (23.21)	38.65 (5.9, 113.0)	43.07 (3.176)	36.83, 49.31	<0.0001
Post-treatment Month 18	33	39.87 (24.08)	35.00 (4.5, 122.9)	38.72 (3.172)	32.49, 44.95	<0.0001
Post-treatment Month 24	33	38.55 (19.19)	35.40 (4.7, 99.2)	37.40 (2.933)	31.64, 43.16	<0.0001

Abbreviations: AAV5 = adeno-associated viral vector serotype 5; aPTT = activated partial thromboplastin time; CI = confidence interval; FIX = Factor IX; LOD = limit of detection; LS = least square; Max = maximum; Min = minimum; NAb = neutralizing antibody; SD = standard deviation; SE = standard error.
Uncontaminated data were used; blood samples did not occur within 5 half-lives of exogenous FIX use. Both the date and time of the exogenous FIX use (start) and the blood sampling were considered in determining contamination. FIX levels beginning with the Week 3 assessment were used in the analysis. Subjects with zero uncontaminated central-laboratory post-AMT-061 values had their change from baseline assigned to zero for this analysis, and had their post-baseline values set equal to their baseline value. "With antibodies" was defined as having a titer of >LOD. "Without antibodies" was defined as having a titer of ≤LOD. Baseline antibody titer was the most recently collected non-missing antibody titer prior to dosing.

1. LS Mean from repeated measures linear mixed model with visit as a categorical covariate.
2. One-sided p-value ≤0.025 for post-treatment >baseline was regarded as statistically significant. For Month 24, p-value not adjusted for multiplicity.
3. Baseline FIX was imputed based on subject's historical hemophilia B severity documented on the case report form. If the subject had documented severe FIX deficiency (FIX plasma level <1%), their baseline FIX activity level was imputed as 1%. If the subject had documented moderately severe FIX deficiency (FIX plasma level ≥1% and ≤2%), their baseline FIX activity level was imputed as 2%.

Both subjects with and without anti-AAV nAbs at baseline responded to treatment with AMT-061, with the exception of one subject with a baseline anti-AAV nAb titre >3000.

However, while baseline FIX levels were equally low in both subgroups, the increase of FIX activity appears to be higher in those subjects without anti-AAV nAbs.

Correlation of FIX Activity Levels at Month 18 with pre-IMP Neutralizing Antibodies to AAV5 Titres

Neutralizing antibodies were present in 21/54 (38.9%) subjects at baseline, prior to AMT-061 treatment. The linear regression indicated a trend to lower mean FIX activity in subjects with anti-AAV5 nAbs at baseline. However, no clinically meaningful correlation between an individual's titre of pre-existing anti-AAV5 nAbs with their FIX activity at 18 months or at 24 months post-treatment was identified up to a nAb titre of 3212.3 (18-month Pearson coefficient: -0.35; Spearman coefficient: -0.30; R2: 0.124; 24-month Pearson coefficient: -0.36; Spearman coefficient: -0.29; R2: 0.129). Additionally, the primary endpoint of ABR was met in both subjects with or without pre-existing anti-AAV5 nAbs at baseline. One subject with a titre of 3212.3 for pre-existing anti-AAV5 nAbs at screening did not respond to treatment with AMT-061, and similar results were observed when this subject was

excluded from the correlation. This subject ended the study early, having withdrawn consent after 24 months post-treatment (Month 24 visit not completed).

Similar results were observed when the arithmetic mean FIX activity across Month 6 to Month 18 or to Month 24 was considered, when subjects with baseline anti-AAV5 nAb titre <3000 were included, and when the correlation of FIX activity with anti-AAV5 nAb titre at the lead-in final visit was assessed.

However, the lack of a statistically significant correlation between the presence of neutralizing antibodies at baseline and mean FIX activity could primarily be an artefact of the small sample size. As the applicant has noted, the linear regression does indeed indicate a trend of lower FIX activity in subjects with anti-AAV5 nAbs at baseline.

Target Joints

A target joint was defined as 3 or more spontaneous bleeding episodes into a single joint within a consecutive 6-month period prior to the dosing visit and which was not resolved by the time of dosing. An identified target joint with ≤2 spontaneous bleeding episodes within a consecutive 12-month period was considered resolved.

At dosing, 2 subjects had pre-existing targets joints, which resolved during the post-treatment period. The time to resolution of target joints for these subjects was 121 and 327 days post-AMT-061 treatment.

One subject had a new target joint (left knee joint) that occurred during the post-treatment period after stable FIX expression on Day 381, which was not resolved at the data cut-off for this report.

The one patient who developed the target joint was the patient with the baseline anti-AAV nAb titre of 3212.3, who was a non-responder to treatment with Hemgenix. No responder developed a new target joint during the available observation period of 24 months.

Subjects with Zero Bleeding Episodes

The number (%) of subjects with zero bleeding episodes increased following treatment with AMT-061, from **14/54 (25.9%)** subjects during the ≥6-month lead-in period to **34/54 (63.0%)** subjects during the Month 7 to 18 post-treatment period. A higher number of subjects **(27/54 [50.0%])** had zero bleeding episodes during the Month 7 to 24 post-treatment period compared to the lead-in period.

For subjects with a *negative baseline anti-AAV5 nAb titre*, **11/33 (33.3%)** subjects had 0 bleeding episodes during the lead-in period and **23/33 (69.7%)** and **19/33 (57.6%)** subjects had 0 bleeding episodes during the Month 7 to 18 and Month 7 to 24post-treatment periods, respectively.

For subjects with a *positive baseline anti-AAV5 nAb titre*, **3/21 (14.3%)** subjects had 0 bleeding episodes during the lead-in period and **11/21 (52.4%)** and **8/21 (38.1%)** subjects had 0 bleeding episodes during Months 7 to 18 and Month 7 to 24 post-treatment periods, respectively.

International Physical Activity Questionnaire

The iPAQ assesses physical activity undertaken across a comprehensive set of domains including leisure time, domestic and gardening (yard) activities, and work and transport-related activity. Based on the repeated measures linear mixed model, the numerical difference in iPAQ scores between the Lead in and Post-treatment Periods was not significant, with a LS mean (SE) difference of -721.2

(528.61; 95% CI: -1770.6, 328.3; p-value = 0.9121). Between 12 and 24 months (i.e., Year 2) post-treatment, the LS mean (SE) difference was -785.8 (553.40; 95% CI: -1896.8, 325.2; p = 0.9191).

EuroQol-5 Dimensions-5 Levels VAS Scores

The EQ-5D-5L descriptive system of health-related QoL states consists of 5 dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression). The EQ-5D-5L questionnaire consists of the EQ-5D-5L descriptive system and the EQ VAS which reflects the patient's perception of their overall health on a scale from 0 to 100. No notable difference between the lead-in and post-treatment periods was observed with a LS mean (SE) difference of 0.1 (1.84; 95% CI: -3.5, 3.8; p-value 0.4753). However, in the second year post-treatment there was a statistically significant improvement in the mean EQ-5D-5L VAS scores with a LS mean (SE) difference between 12 and 24 months (i.e., Year 2) post-treatment of 2.8 (1.40; 95% CI: 0.0, 5.6; p = 0.0244 [not adjusted for multiplicity]).

In the IPAQ and EQ-5D-5L scores, at 18 months post treatment no improvement could be observed. At 24 months, the IPAQ did not show significant changes, but the EQ-5Q-5L score could detect an improvement between year 1 and year 2. Most subjects already received state of the art FIX prophylaxis with extended half-life products, therefore the burden of infusions is relatively low and it is more difficult to demonstrate an additional beneficial effect.

Exploratory Efficacy Measures

FIX protein levels

During the 18 months post-AMT-061 treatment, FIX protein levels fluctuated across visits and ranged from 19.35% to 25.25%. FIX protein levels during the post-treatment period followed a similar trend to FIX activity by one-stage (aPTT-based) activity; however, more variability was observed in the protein concentrations.

The mean ratio of uncontaminated FIX activity to protein was 5.867 at Week 3, increasing to 8.078 at Week 10. The mean ratio of FIX activity to FIX protein level was stable at approximately 7 to 8.5 between Month 6 and Month 18.

Haemophilia Joint Health Score

The HJHS measures joint health, in the domain of body structure and function (i.e., impairment), of the joints most commonly affected by bleeding in haemophilia: the knees, ankles, and elbows. The total score ranges from 0 to 124, with higher scores considered unfavourable.

Mean (SD) HJHS at screening was 20.8. At the end of the lead-in period, mean (SD) HJHS in the FAS was 21.2 (16.9; Table 21). Following 12 months of treatment with AMT-061, mean (SD) HJHS was 19.5 (16.8). The LS mean (SE) difference in HJHS score between the lead-in and post-treatment periods was -1.7 (0.79; 95% CI: -3.3, -0.1; p-value: 0.0196 [not adjusted for multiplicity]).

A minimal improvement in the HJHS could be detected. However, the HJHS is primarily designed for children with haemophilia aged 4-18 years with mild joint impairment and has not yet been adequately studied for use in adults or more severe joint disease, therefore these outcomes have to be interpreted with caution.

Rate of Traumatic Bleeding Episodes

During the lead-in period, 29/54 (53.7%) subjects experienced 70 traumatic bleeding episodes. During the Month 7 to 18 post-treatment period, there were 30 traumatic bleeding episodes in 12/54 (22.2%) subjects, including 16 episodes in 9 (16.7%) subjects during the Month 7 to 12 period and 14 episodes in 7 (13.0%) subjects during the Month 13 to 18 period.

The mean number of traumatic bleeding episodes per subject decreased following treatment with AMT-061 (1.3 during lead-in period to 0.3 during both the Month 7 to 12 and Month 13 to 18 post-treatment period). The ABR of traumatic bleeding episodes decreased following AMT-061 treatment, from 2.09 (95% CI: 1.42, 3.08) for the lead-in period to 0.62 (95% CI: 0.31, 1.23) for the Month 7 to 18 post-treatment period.

Traumatic bleeding episodes treated with FIX included 58 episodes in 26/54 (48.1%) subjects during the lead-in period and 11 episodes in 9/54 (16.7%) subjects during the Month 7 to 18 period. For FIX-treated traumatic bleeding episodes, the ABR decreased from 1.74 (95% CI: 1.21, 2.49) for the lead-in period to 0.22 (95% CI: 0.11, 0.45) for the Month 7 to 18 post-treatment period.

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

The mean (SD) PROBE summary scores were similar between screening (0.778 [0.161]) and the end of the lead-in period (0.787 [0.166]). The mean (SD) PROBE summary score was 0.811 (0.168) at Month 12 post-treatment with AMT-061. The mean PROBE scores for males and females without bleeding disorders were reported as 0.909 and 0.869 respectively. While there still appears to be a decrement in QoL compared to subjects with no bleeding disorders, the mean scores in AMT-061-treated subjects were higher in the post-treatment period compared to the lead-in period.

More research is needed in the future to ascertain what constitutes a clinically meaningful change in PROBE scores with a therapeutic intervention.

Work Productivity and Activity Impairment

The WPAI assesses the effect of health problems on a subjects' ability to work and perform regular activities. It consists of 4 domains including absenteeism (defined as the percent of time missed work due to health problems), presenteeism (defined as percent impairment while working), work productivity loss (which is a combination of absenteeism and presenteeism), and activity impairment.

During the lead-in period, mean absenteeism in the FAS was 4.97%, 5.95%, and 4.54% at the baseline, Month 4, and final lead-in period visits, respectively. During the post-treatment period, mean absenteeism was 6.45% at baseline and then was 0.51% and 0.71% at the post-treatment Month 6 and Month 12 visits, respectively. During the lead-in period, presenteeism was 21.16%, 19.71%, and 16.06% at the baseline, Month 4, and the final lead-in period visits, respectively. During the post-treatment period, baseline mean presenteeism was 17.61% and was 11.35% and 10.98% at the post-treatment Month 6 and Month 12 visits, respectively. Activity impairment levels were similar between the lead-in and post-treatment periods.

Brief Pain Inventory

The BPI evaluates the severity of a subject's pain and the impact of this pain on the subject's daily functioning. Pain was assessed on a scale of 0 (no pain) to 10 (pain as bad as you can image), and the level of pain interference with various activities was assessed on a scale of 0 (did not interfere) to 10 (completely interfered).

Based on the repeated measures linear mixed model, controlling for the effect of period, visit, and period-by-visit interaction, pain intensity decreased numerically following treatment with AMT-061, with a LS mean (SE) difference of -0.25 (0.143; 95% CI: -0.53, 0.04; p-value 0.0431 [not adjusted for multiplicity].

The numerical decrease in pain interference scores between the lead-in and post-treatment periods was not significant, with a LS mean (SE) difference of -0.21 (0.161; 95% CI: -0.52, 0.11; p-value 0.1023 [not adjusted for multiplicity].

Haemophilia Activities List

The HAL measures the impact of haemophilia on self-perceived functional abilities within 8 modules. The difficulty due to haemophilia in the previous month for the domains of each module were assessed as either: impossible, always a problem, mostly a problem, sometimes a problem, rarely a problem, or never a problem.

Based on the repeated measures linear mixed model controlling for the effect period, visit, and period-by-visit interaction, the numerical difference in HAL scores between the lead-in and post-treatment periods was not significant with a LS mean (SE) difference of 1.16 (1.287; 95% CI: -1.38, 3.71; p-value 0.1843 [not adjusted for multiplicity];

Haemophilia Specific Quality of Life Index

The Hem-A-QoL captures aspects of QoL for adult subjects with hemophilia within 10 domains. The response options for each question were never, rarely, sometimes, often, or all the time. Scores ranged from 0 to 100; lower Hem-A-QoL scores represent a better QoL and higher scores are indicative of lower QoL.

A one-sided p-value ≤0.025 for the post-treatment vs lead-in period was considered statistically significant. The analyses were not adjusted for multiplicity. Significant model-based mean differences in scores compared with the lead-in period were noted for the Total Score (LS mean -5.50; <0.0001), and were also noted for the domains "Treatment" (LS mean -14.88; p<0.0001), "Feelings" (LS mean -9.42; p<0.0001), "Future" (LS mean -5.02; p = 0.0023), and "Work/School" (LS mean -4.99; p = 0.0036).

EuroQol-5 Dimensions-5 Levels Index Scores

The EQ-5D-5L descriptive system of health-related QoL states on which the index scores are based consist of 5 domains including mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.

Based on the repeated measures linear mixed model, controlling for the effect of period, visit, and period-by-visit interaction, there was a numerical improvement in EuroQol index scores in the post-treatment period compared to the lead-in period, but it was not statistically significant at the p = 0.025 threshold. The LS mean (SE) was 0.0310 (0.01903; 95% CI: -0.0067, 0.0686; p-value: 0.0530 [not adjusted for multiplicity]).

Minimal or no improvements could be detected in the WPAI, BPI, HAL and EQ-5D-5L.

A minimal clinically important improvement was identified as a 10-point reduction in the 'Physical Health' and 'Sports & Leisure' domains, and a 7-point reduction in 'Total Score' for the **Hem-A-QoL** in Wyrwich et al, Haemophilia. 2015 Sep;21(5):578-84. Therefore, while the reported improvements were considered statistically significant, their clinical relevance is borderline, which is in line with other outcomes reported from PRO endpoints.

As already mentioned with regard to the secondary endpoints IPAQ and EQ-5D-5L, most patients received prophylaxis with EHL FIX products, and it is difficult to demonstrate increased benefit as the burden of treatment is relatively low, with FIX infusions necessary every 7-14 days. However, it is possible that the significantly decreased bleeding rate could lead to improved PRO scores at later observation time-points as e.g. joint health will be preserved in the long-term.

- **Ancillary analyses**

Table 12. Subgroup Analysis of Annualised Bleeding Rates (Full Analysis Set)

	≥6-month Lead-in Period[1]		Month 7-18[1]			Month 7-24[1]		
	Unadjusted ABR	Adjusted ABR (95% CI)	Unadjusted ABR	Adjusted ABR (95% CI)	Rate Ratio (Two-sided 95% CI) p-value[2,3]	Unadjusted ABR	Adjusted ABR (95% CI)	Rate Ratio (Two-sided 95% CI) p-value[2,3]
Age Group								
<40 (N = 31)	3.84	4.09 (2.89, 5.80)	1.31	2.33 (0.84, 6.45)	0.57 (0.21, 1.51) 0.1278	1.24	1.83 (0.81, 4.13)	0.45 (0.21, 0.95) 0.0184
40 to <60 (N = 15)	5.02	4.95 (3.24, 7.57)	0.75	0.78 (0.26, 2.31)	0.16 (0.06, 0.39) <0.0001	0.50	0.52 (0.17, 1.52)	0.10 (0.04, 0.26) <0.0001
≥60 (N = 8)	3.29	NC	0.82	NC	NC	1.00	NC	NC
Race Group								
White (N = 40)	3.58	3.57 (2.50, 5.11)	0.89	0.94 (0.47, 1.89)	0.26 (0.15, 0.47) <0.0001	0.87	0.92 (0.50, 1.71)	0.26 (0.15, 0.43) <0.0001
Non-White or Not Specified (N = 14)	5.70	5.88 (4.26, 8.13)	1.74	53.75 (8.56, 337.51)	9.14 (1.37, 60.91) 0.0111	1.39	58.02 (9.90, 340.12)	9.90 (1.59, 61.73) 0.0070
Ethnic Group								
Hispanic or Not Specified (N = 9)	5.50	NC	1.69	NC	NC	1.60	NC	NC
Not Hispanic or Latino (N = 45)	3.85	3.94 (2.84, 5.46)	0.96	1.66 (0.72, 3.80)	0.42 (0.19, 0.92) 0.0154	0.87	1.78 (0.78, 4.04)	0.45 (0.21, 0.99) 0.0241
Lead-in Bleeding Episode Count Category								
≥1 (N = 40)	NC	NC	1.19	NC	NC	1.06	NC	NC
0 (N = 14)	NC	NC	0.79	NC	NC	0.78	NC	NC
Status of Target Joint at Screening Category								
Absence (N = 44)	3.42	3.16 (2.36, 4.22)	0.68	0.77 (0.43, 1.36)	0.24 (0.13, 0.44) <0.0001	0.71	0.88 (0.52, 1.47)	0.29 (0.16, 0.52) <0.0001
Presence (N = 10)	7.24	7.89 (5.25, 11.84)	3.06	105.84 (15.97, 701.57)	13.42 (1.75, 102.96) 0.0062	2.32	109.31 (16.38, 729.52)	13.91 (1.79, 108.18) 0.0060
Baseline anti-AAV5 NAb Titer Category								
Negative (N = 33)	3.76	3.79 (2.55, 5.63)	0.90	0.93 (0.44, 1.98)	0.25 (0.14, 0.43) <0.0001	0.79	0.80 (0.39, 1.67)	0.21 (0.12, 0.37) <0.0001
Positive (N = 21)	4.64	4.97 (3.66, 6.75)	1.41	8.77 (1.97, 39.06)	1.77 (0.41, 7.62) 0.2232	1.37	12.59 (2.95, 53.66)	2.56 (0.61, 10.66) 0.0986
Baseline anti-AAV5 NAb Titer								

<3000 (N = 53) 4.17	3.89 (2.93, 5.16)	0.98	1.07 (0.63, 1.82)	0.28 (0.17, 0.43) <0.0001	0.93	1.09 (0.67, 1.79)	0.28 (0.17, 0.46) <0.0001
Baseline HIV Category							
Negative (N = 51) 3.95	4.06 (3.07, 5.36)	1.11	1.62 (0.84, 3.14)	0.40 (0.22, 0.73) 0.0015	1.01	1.66 (0.87, 3.19)	0.41 (0.22, 0.75) 0.0020
Positive (N = 3) 6.71	NC	0.67	NC	NC	0.67	NC	NC
Hepatitis B or C Category							
Yes (N = 33) 3.61	3.77 (2.69, 5.28)	0.81	1.55 (0.60, 4.04)	0.41 (0.17, 1.02) 0.0272	0.70	3.43 (1.02, 11.50)	0.92 (0.28, 2.98) 0.4426
No (N = 21) 4.90	4.88 (3.26, 7.31)	1.48	1.56 (0.74, 3.28)	0.32 (0.18, 0.58) <0.0001	1.41	1.47 (0.75, 2.87)	0.30 (0.17, 0.52) <0.0001
Baseline Fibrosis Test Score Category <9 kPa (N = 54) 4.11	4.19 (3.22, 5.45)	1.08	1.51 (0.81, 2.82)	0.36 (0.20, 0.64) 0.0002	0.99	1.51 (0.83, 2.76)	0.36 (0.21, 0.63) 0.0002
Baseline Steatosis Grade Category							
<S2 (N = 28) 3.72	4.06	0.99	2.01	0.49	0.99	1.57	0.39
	(2.83, 5.82)		(0.59, 6.84)	(0.15, 1.59)		(0.59, 4.16)	(0.16, 0.95)
				0.1191			0.0192
≥S2 (N = 12) 4.55	3.42	1.05	1.44	0.42	0.89	2.77	0.77
	(2.03, 5.76)		(0.52, 4.00)	(0.14, 1.24)		(0.71, 10.90)	(0.19, 3.11)
				0.0588			0.3554
Missing (N = 14) 4.47	4.41	1.30	1.36	0.31	1.08	1.15	0.26
	(2.54, 7.67)		(0.60, 3.08)	(0.16, 0.60)		(0.58, 2.26)	(0.14, 0.49)
				0.0002			<0.0001

Abbreviations: AAV5 = adeno-associated viral vector serotype 5; ABR = annualised bleeding rate; CI = confidence interval; HIV = human immunodeficiency virus; NAb = neutralizing antibody; NC = not calculated.
When n <10 and for subgroups based on "Lead-In Bleed Count Category", model-based statistics were not calculated. No Lead-In-Period statistics were provided for subgroups based on "Lead-In Bleed Count Category".

[1.] Adjusted ABR and comparison of ABR between lead-in and post-treatment period was estimated from a repeated measures generalized estimating equations negative binomial regression model accounting for the paired design of the trial with an offset parameter to account for the differential collection periods. Treatment period was included as a categorical covariate. Lead-in period data for the Month 7 to 24 comparisons not shown in table; these data are available in the outputs listed below as Source for Month 7 to 24.

[2.] The upper limit of the confidence interval of the rate ratio was compared to the non-inferiority margin of 1.8. If the upper limit was less than 1.8, then non-inferiority was declared.

[3.] One-sided p-value ≤0.025 for post-treatment/lead-in <1 was regarded as statistically significant p- values not adjusted for multiplicity.

ABR was significantly reduced during Months 7 to 18 after AMT-061 treatment compared to the lead-in period for most of the subgroups analysed, with rate ratios (post-treatment/lead-in) ranging from 0.16 to 0.57 (p <0.025 for most subgroups (not adjusted for multiplicity). Exceptions to this included subjects with a positive anti-AAV5 nAb titre at baseline (N = 21; rate ratio = 1.77), non-White subjects (N = 14; rate ratio = 9.14), and subjects who had target joints at screening (N = 10; rate ratio = 13.42); however, for these subgroups, the unadjusted ABR at Months 7 to 18 post-treatment was less than that for the lead-in period. In the ABR analysis, all bleeding episodes were counted but person-time during the post-treatment period (on any day that began) within 5 half-lives subsequent to exogenous FIX use was not considered to be time at risk of (having) a bleeding episode, which may have led to higher subject estimates for some subgroups.

With the responses to the D120 LoQ, the applicant provided an ABR subgroup analysis for those subjects who required corticosteroid treatment due to elevated transaminases. The ABR is comparable to that of subjects who did not require corticosteroid treatment and significantly reduced compared to the run-in period.

Table 13. Annualised Bleeding Rate by Subgroups of Transaminitis Treatment With Corticosteroids – Months 7 to 18 Post-treatment Period (Full Analysis Set)

Endpoint	≥ 6-month Lead-in Period	Months 7-18 Post-treatment Period						Conclusion
	Unadjusted ABR [a] (Mean No. of Bleeds)	Adjusted ABR (95% CI) [b]	Unadjusted ABR [a]	Adjusted ABR (95% CI) [b]	Rate ratio (Post-treatment / Lead-in) [b]	Two-sided 95% Wald CI [c]	p-value [d]	
All bleeding episodes (subjects with transaminase elevations treated with corticosteroids; N = 9)	3.61 (2.2)	3.76 (2.34, 6.02)	0.82	0.83 (0.28, 2.42)	0.22	0.09, 0.57	0.0008	NI met SUP met
All bleeding episodes (subjects with no transaminase elevations treated with corticosteroids; N = 45)	4.21 (2.6)	4.27 (3.17, 5.76)	1.14	1.85 (0.87, 3.92)	0.43	0.21, 0.88	0.0100	NI met SUP met

Table 14. Subgroup Analysis of FIX Activity at 18 Months Post-AMT-061 Administration (One-stage [aPTT-based] Assay; Full Analysis Set)

	Baseline[1]	Change from Baseline to Month 18			Change from Baseline to Month 24		
	Mean (SD)	Mean (SD)	LS Mean (SE)[2]	95% CI p-value[3]	Mean (SD)	LS Mean (SE)[2]	95% CI p-value[3]
Age Group							
<40 (N = 31)	1.16 (0.37)	29.06 (14.27)	27.66 (2.495)	22.76, 32.56	30.13 (15.74)	28.76 (2.478)	23.89, 33.63
				<0.0001			<0.0001
40 to <60 (N = 15)	1.27 (0.46)	38.05 (15.82)	38.05 (4.527)	29.13, 46.96	41.10 (19.33)	41.10 (4.513)	32.21, 49.99
				<0.0001			<0.0001
≥60 (N = 8)	1.13 (0.35)	62.12 (39.55)	NC	NC	47.27 (26.08)	NC	NC
Race Group							

White (N = 40)	1.20 (0.41)	37.89 (23.05)	37.08 (2.925)	31.34, 42.83	37.74 (20.06)	36.95 (2.782)	31.48, 42.41
				<0.0001			<0.0001
Non-White or Not	1.14 (0.36)	28.86 (14.08)	26.35 (4.117)	18.23, 34.48	28.31 (13.52)	26.09 (3.909)	18.38, 33.80
Specified (N = 14)				<0.0001			<0.0001
Ethnic Group							
Hispanic or Not Specified (N = 9)	1.22 (0.44)	25.81 (14.81)	NC	NC	25.31 (13.03)	NC	NC
Not Hispanic or Latino (N = 45)	1.18 (0.39)	37.61 (22.14)	36.52 (2.751)	31.12, 41.92 <0.0001	37.41 (19.47)	36.35 (2.643)	31.16, 41.54 <0.0001
Lead-in Bleeding Episode Category							
≥1 (N = 40) 1.18 (0.38)		33.89 (19.92)	32.67 (2.461)	27.84, 37.50	32.35 (13.80)	31.13 (2.360)	26.50, 35.76
				<0.0001			<0.0001
0 (N = 14) 1.21 (0.43)		40.93 (25.52)	38.92 (6.860)	25.39, 52.45	44.37 (28.09)	42.32 (7.007)	28.51, 56.13
				<0.0001			<0.0001
Status of Target Joint at Screening Category							
Absence (N = 44) 1.18 (0.39)		36.29 (23.03)	34.99 (2.795)	29.50, 40.48 <0.0001	35.98 (20.11)	34.84 (2.611)	29.71, 39.96 <0.0001
Presence (N = 10) 1.20 (0.42)		33.13 (12.72)	NC	NC	33.20 (13.58)	NC	NC
Baseline anti-AAV5 NAb Titer Category							
Negative (N = 33)	1.15 (0.36)	38.72 (24.16)	38.72 (3.172)	32.49, 44.95	37.40 (19.27)	37.40 (2.933)	31.64, 43.16
				<0.0001			<0.0001
Positive (N = 21)	1.24 (0.44)	29.90 (13.74)	26.83 (3.854)	19.24, 34.41	31.75 (18.49)	28.35 (3.929)	20.62, 36.08
				<0.0001			<0.0001
Baseline anti-AAV5 NAb Titer							
<3000 (N = 53)	1.19 (0.39)	35.72 (21.46)	34.66 (2.434)	29.88, 39.44	35.48 (19.01)	34.45 (2.311)	29.92, 38.99
				<0.0001			<0.0001
Baseline HIV Category							
Negative (N = 51)	1.20 (0.40)	36.25 (21.68)	34.74 (2.520)	29.79, 39.68	35.96 (19.18)	34.49 (2.418)	29.75, 39.24
				<0.0001			<0.0001
Positive (N = 3)	1.00 (0.00)	27.47 (19.17)	NC	NC	27.90 (17.39)	NC	NC
Hepatitis B or C Category							
Yes (N = 33)	1.15 (0.36)	40.25 (24.50)	38.71 (3.476)	31.88, 45.54	39.05 (20.86)	37.40 (3.377)	30.76, 44.03
				<0.0001			<0.0001
No (N = 21)	1.24 (0.44)	28.94 (13.84)	27.71 (2.891)	22.03, 33.40	30.12 (14.75)	28.95 (2.728)	23.58, 34.31
				<0.0001			<0.0001
Baseline Fibrosis Test Score Category							
<9 kPa (N = 54)	1.19 (0.39)	35.72 (21.46)	34.31 (2.444)	29.52, 39.11	35.48 (19.01)	34.13 (2.325)	29.57, 38.69
				<0.0001			<0.0001
Baseline Steatosis Grade Category							

<S2 (N = 28)	1.11 (0.31)	40.46 (25.79)	39.72 (3.717)	32.42, 47.03	39.04 (19.84)	38.25 (3.607)	31.16, 45.34
				<0.0001			<0.0001
≥S2 (N = 12)	1.25 (0.45)	28.02 (13.89)	23.42 (4.726)	14.08, 32.76	27.18 (13.30)	22.98 (4.580)	13.93, 32.03
				<0.0001			<0.0001
Missing (N = 14)	1.29 (0.47)	31.81 (13.37)	32.22 (3.840)	24.65, 39.79	34.45 (20.09)	34.96 (4.302)	26.48, 43.43
				<0.0001			<0.0001
Any Post-Treatment use of Systemic Corticosteroid for Transaminitis Category							
No (N = 45) 1.18 (0.39)		40.42 (20.65)	38.33 (2.638)	33.15, 43.51	40.12 (17.55)	38.09 (2.511)	33.16, 43.02
				<0.0001			<0.0001
Yes (N = 9) 1.22 (0.44)		14.33 (7.89)	NC	NC	14.30 (7.65)	NC	NC

Abbreviations: AAV5 = adeno-associated viral vector serotype 5; aPTT = activated Partial Thromboplastin Time; CI = confidence interval; HIV = human immunodeficiency virus; FIX = Factor IX; LS = least squares; NAb = neutralizing antibody; NC = not calculated (because timepoints with n <10 were excluded from the modeling); SD = standard deviation; SE = standard error. Measurement of FIX activity did not occur within 5 half-lives of exogenous FIX use. Subjects with central-laboratory post-AMT-061 values in this timeframe had their change from baseline assigned to zero for this analysis.

[1.] Baseline FIX was imputed based on subject's historical hemophilia B severity documented on the case report form. If the subject had documented severe FIX deficiency (FIX plasma level <1%), their baseline FIX activity level was imputed as 1%. If the subject had documented moderately severe FIX deficiency (FIX plasma level ⩾1% and ⩽2%,) their baseline FIX activity level was imputed as 2%.

[2.] LS mean from repeated measures linear mixed model with visit as a categorical covariate. Lead-in period data for the Month 7 to 24 comparisons not shown in table; these data are available in the output listed below as Source for Month 7 to 24.

[3.] One-sided p-value ⩽0.025 for post-treatment >baseline was regarded as statistically significant. p-values not adjusted for multiplicity.

For all subgroups, calculated FIX activity was significantly higher at Month 18 post-AMT-061 administration compared to baseline (p <0.0001, not adjusted for multiplicity). Mean FIX activity ranged between 1.00% and 1.29% at baseline and LS mean increases from baseline ranged between 23.42% and 39.72% of normal across subgroups. Numerical differences within some subgroups were noted, especially for the subgroup of 9 subjects who received systemic corticosteroids post-AMT-061 treatment. This subgroup achieved the lowest mean FIX activity levels at 14.33%. All other subgroups showed approximately double or higher FIX activity.

Effect of Intrinsic and Extrinsic Factors on FIX Activity

Effect of intrinsic and extrinsic factors on uncontaminated FIX activity was evaluated in the pharmacokinetic (PK) population, defined as subjects receiving a full dose of etranacogene dezaparvovec and have at least 1 post dose FIX activity measurement in Study CT-AMT-061-02.

A trend of higher mean FIX activity with increase in age was observed. Whilst there were differences in mean FIX activity levels between the subgroups, especially between the < 40 years and ≥ 60 years of age subgroups, the impact of age on FIX activity as an independent variable cannot be established. The mean FIX activity for the < 40 years of age subgroup was > 30% of normal activity at Month 6 to 18, and all subjects in this subgroup achieved a FIX activity level within the mild to non-haemophilia B range; the minimum FIX activity level at Month 6 to 18 was at least 8%.

Subjects with mild renal impairment (N = 7/53; PK population) had slightly higher mean FIX activity (up to 37% relative difference) compared to those with normal renal function during Month 6 to 18 post dose. One subject with moderate renal impairment in the study had similar FIX activity as subjects with normal renal function. The impact of moderate renal impairment, severe renal impairment, and end stage renal disease on FIX activity could not be fully assessed due to either

limited (“moderate”) or no (“severe”, and “end stage renal disease”) subject representation of these subgroups.

Subjects with steatosis Controlled Attenuation Parameter (CAP) scores of ≥ S2 (≥ 260 decibels / meter [dB/m]), < S2 (< 260 dB/m) and missing score showed no clinically meaningful difference in the mean FIX activity levels. Evaluation of the impact of race, ethnicity, body mass index, and baseline FIX activity at the time of historical diagnosis on FIX activity showed that all subgroups within each of these variables had clinically meaningful increases in FIX activity post dose.

Thirteen out of 53 subjects who received a full dose of etranacogene dezaparvovec experienced alanine aminotransferase (ALT) elevation (ALT > upper limit of normal [ULN] when the baseline ALT is below ULN, or ALT > 2 × baseline value, occurring over the initial 90 days post dose) and 9 subjects were treated with corticosteroids for ALT elevation of either > ULN (n = 8) or > 2 × baseline value (n = 1). Subjects with ALT elevation had approximately 44% lower mean FIX activity at Month 18 compared to those that did not have ALT elevation. The 9/53 subjects that were treated with corticosteroid for ALT elevations exhibited approximately 63% lower mean FIX activity at Month 18 compared to those who did not receive corticosteroid co-administration. The mean FIX activity in the limited number of subjects (n = 9) treated with corticosteroids for ALT elevations was > 15% of normal and mean FIX activity levels remained in the mild hemophilia B range across all time points.

Different drug product batches used in Study CT-AMT-061-02 showed no notable differences in the mean FIX activity at 6, 12, and18 months after etranacogene dezaparvovec administration.

Due to the small number of subjects in each subgroup, outcomes have to be interpreted with caution.

The subgroup of subjects demonstrating appreciably lower FIX activity levels was comprised of those patients who experienced an ALT elevation post AMT-061 treatment and especially those patients who received corticosteroids as a consequence. The highest FIX activity during months 7-18 in a patient treated with corticosteroids was ~30% and the lowest only 4.5%.

Summary of main efficacy results

The following table summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 15. Summary of Efficacy Study 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.		
Study identifier	NCT03569891 EudraCT number: 2017-004305-40	
Design	CT-AMT-061-02 is an ongoing Phase III, open-label, single-dose, multi-center, multi-national trial, with a screening phase / period, a lead-in phase / period, a treatment plus a post-treatment follow-up phase / period, and a long-term follow-up phase / period.	
	Duration of main phase: Duration of run-in phase: Duration of extension phase:	1 day (single dose) Variable length; minimum of 6 months 5 years: 52-week post-treatment follow-up phase and 4-year long-term follow-up phase.
Hypothesis	Primary endpoint: 1-sided noninferiority	

Treatments group	AMT-061	Subjects were planned to receive a single intravenous infusion of 2×10^{13} gc/kg AMT-061. 67 subjects enrolled.
Key endpoints and definitions	Primary	Annualised bleeding rate comparison between AMT-061 and prophylaxis for noninferiority between the lead-in phase and the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment).
	Secondary	Endogenous FIX activity at 6 months after AMT-061 dosing.
	Secondary	Endogenous FIX activity at 12 months after AMT-061 dosing.
	Secondary	Endogenous FIX activity at 18 months after AMT-061 dosing.
	Secondary	Annualised consumption of FIX replacement therapy during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment), excluding FIX replacement for invasive procedures, compared to the lead-in phase.
	Secondary	Annualised infusion rate of FIX replacement therapy during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment), excluding FIX replacement for invasive procedures, compared to the lead-in phase.
	Secondary	Proportion of subjects remaining free of previous continuous routine prophylaxis during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment).
	Secondary	Annualised bleeding rate comparison between AMT-061 and prophylaxis for superiority between the lead-in phase and the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment).
	Secondary	Rate of spontaneous bleeding episodes during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) compared to the lead-in phase.
	Secondary	Rate of joint bleeding episodes during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) compared to the lead-in phase.
	Secondary	Estimated ABR – during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment) – as a function of pre-IMP anti-AAV5 antibody titres using the luciferase based NAb assay (as a "correlation" analysis).
	Secondary	Proportion of subjects with zero bleeding episodes during the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment).
Database lock	25 January 2022	
Results and Analysis The primary and secondary clinical efficacy endpoints were met, demonstrating the superiority of etranacogene dezaparvovec (AMT-061) over standard of care in the treatment of hemophilia B.		

Analysis Description	**Primary Analysis**				
Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
		All bleeding episodes		All bleeding episodes for subjects with anti-AAV5 NAb < 3000	
		≥ 6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)	≥ 6-month Lead-in Period (N = 53)	Month 7-18 (N = 53)
	Adjusted ABR (95% CI)	4.19 (3.22, 5.45)	1.51 (0.81, 2.82)	3.89 (2.93, 5.16)	1.07 (0.63, 1.82)
	Rate ratio (post-treatment / lead-in)	-	0.36	-	0.28
	Two-sided 95% Wald CI	-	0.20, 0.64	-	0.17, 0.43
	p-value (1-sided p-value ≤ 0.025 for post-treatment / lead-in < 1 was regarded as statistically significant)	-	0.0002	-	< 0.0001
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Endogenous FIX Activity at 6 Months, 12 Months, and 18 Months**				
Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
	Endogenous FIX activity	Month 6 (n = 51)	Month 12 (n = 50)	Month 18 (n = 50)	
	Mean (SD)	38.95 (18.72)	41.48 (21.71)	36.90 (21.40)	
	Change from baseline				
	LS mean (SE)	36.18 (2.432)	38.81 (2.442)	34.31 (2.444)	
	95% CI	31.41, 40.95	34.01, 43.60	29.52, 39.11	
	p-value (1-sided p-value ≤ 0.025 for post-treatment / lead-in < 1 was regarded as statistically significant)	< 0.0001	< 0.0001	< 0.0001	
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Annualised Consumption of FIX Replacement Therapy**				

Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
	Annualised exogenous FIX consumption (IU/year)	≥ 6-month Lead-in Period (N = 54)	Post-treatment Period		
			Month 0-6 (N = 54)	Month 7-12 (N = 54)	Month 13-18 (N = 54)
	Unadjusted mean (SD)	257,338.8 (149,013.1)	12,912.9 (37,093.1)	8399.1 (29,720.9)	8486.6 (28,770.2)
	Post-treatment Period – Lead-in Period Differences				
	Adjusted mean (SE)	-	-244,425.8 (19,522.01)	-248,825.0 (21,101.84)	-246,807.0 (20,314.56)
	95% CI	-	-283,582.0, -205,269.6	-291,149.9, -206,500.1	-287,552.9, -206,061.2
	p-value (1-sided p-value ≤ 0.025 for post-treatment – lead-in < 0 was regarded as statistically significant)	-	< 0.0001	< 0.0001	< 0.0001
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Annualised Infusion Rate of FIX Replacement Therapy**				
Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
	Annualised use of FIX replacement therapy (infusions/year)	≥ 6-month Lead-in Period	Post-treatment Period		
			Month 0-6	Month 7-18	Year 0-1
	Number of subjects using FIX replacement therapy, n (%)	54 (100.0)	14 (25.9)	10 (18.5)	11 (20.4)
	Mean number of infusions of FIX replacement therapy (per subject)	44.1	1.6	1.3	1.2
	Adjusted annualised infusion rate (95% CI)	72.49 (63.52, 82.71)	-	2.53 (0.92, 6.96)	3.04 (1.14, 8.12)
	Rate ratio (post-treatment / lead-in)	-	-	0.03	0.04

	Two-sided 95% Wald CI	-	-	0.01, 0.10	0.02, 0.11
	p-value (1-sided p-value ≤ 0.025 for post-treatment / lead-in < 1 was regarded as statistically significant)	-	-	< 0.0001	< 0.0001
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Proportion of Subjects Remaining Free of Previous Continuous Routine FIX Prophylaxis**				
Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
	Portion of subjects remaining free of previous continuous routine FIX prophylaxis (Months 7-18 post-treatment), n (%)	52 (96.3%)			
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Annualised Bleeding Rate – Superiority Assessment**				
Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
	ABR	All bleeding episodes (N = 54)	FIX-treated Bleeding Episodes (N = 54)	All bleeding episodes for subjects with anti-AAV5 NAb < 3000 (N = 53)	
	Rate ratio (Month 7-18 / ≥ 6-month Lead-in Period)	0.36	0.23	0.28	
	Two-sided 95% Wald CI	0.20, 0.64	0.12, 0.46	0.17, 0.43	
	p-value (1-sided p-value ≤ 0.025 for post-treatment / lead-in < 1 was regarded as statistically significant)	0.0002	< 0.0001	< 0.0001	
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Rate of Spontaneous Bleeding Episodes**				

Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
		Spontaneous bleeding episodes		Spontaneous bleeding episodes, FIX-treated	
		≥ 6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)	≥ 6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)
	Number of subjects with a bleeding episode, n (%)	24 (44.4)	9 (16.7)	22 (40.7)	6 (11.1)
	Adjusted ABR (95% CI)	1.52 (1.01, 2.30)	0.44 (0.17, 1.12)	1.34 (0.87, 2.06)	0.45 (0.15, 1.39)
	Rate ratio (Month 7-18 / ≥ 6-month Lead-in Period)	-	0.29	-	0.34
	Two-sided 95% Wald CI	-	0.12, 0.71	-	0.11, 1.00
	p-value (1-sided p-value ≤ 0.025 for post-treatment / lead-in < 1 was regarded as statistically significant)	-	0.0034	-	0.0254
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Rate of Joint Bleeding Episodes**				
Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
		Joint bleeding episodes		Joint bleeding episodes, FIX-treated	
		≥ 6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)	≥ 6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)
	Number of subjects with a bleeding episode, n (%)	32 (59.3)	11 (20.4)	31 (57.4)	9 (16.7)
	Adjusted ABR (95% CI)	2.35 (1.74, 3.16)	0.51 (0.23, 1.12)	2.13 (1.58, 2.88)	0.44 (0.19, 1.00)
	Rate ratio (Month 7-18 / ≥ 6-month Lead-in Period)		0.22		0.20
	Two-sided 95% Wald CI		0.10, 0.46		0.09, 0.45
	p-value (1-sided p-value ≤ 0.025 for post-treatment		< 0.0001		< 0.0001

	/ lead-in < 1 was regarded as statistically significant)				
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Correlation of FIX Activity Levels at Month 18 with Predose Anti-AAV5 NAb Titres**				
Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
	Number (%) of subjects with detectable anti-AAV5 NAbs at baseline	21/54 (38.9%)			
	The linear regression indicated a trend to lower mean FIX activity in subjects with anti-AAV5 NAbs at baseline. However, no clinically meaningful correlation between an individual's titre of preexisting anti-AAV5 NAbs with their FIX activity at 18 months post dose was identified up to a NAb titre of 3212.3 (Pearson coefficient: -0.35; Spearman coefficient: -0.30; R2: 0.124. The primary endpoint of ABR was met in both subgroups, with or without preexisting anti-AAV5 NAbs at baseline. One subject with a titre of 3212.3 for preexisting anti-AAV5 NAbs at screening did not respond to treatment with etranacogene dezaparvovec, and similar results were observed when this subject was excluded from the correlation analysis.				
Effect estimate per comparison	There are no treatment group comparisons for this study.				
Analysis Description	**Secondary Analysis: Subjects with Zero Bleeding Episodes**				
Analysis population and time point description	FAS; ≥ 6-month Lead-in Period / Month 7-18 Post-treatment				
Descriptive statistics and estimate variability	Treatment group	AMT-061			
	Number of subjects (FAS)	54			
		All bleeding episodes		All bleeding episodes for subjects with anti-AAV5 NAb < 3000	
		≥ 6-month Lead-in Period (N = 54)	Month 7-18 (N = 54)	≥ 6-month Lead-in Period (N = 53)	Month 7-18 (N = 53)
	Number of subjects with zero bleeding episodes, n (%)	14 (25.9)	34 (63.0)	13 (24.5)	34 (64.2)
Effect estimate per comparison	There are no treatment group comparisons for this study.				

2.6.5.3. Clinical studies in special populations

Table 16. Clinical Studies in Special Populations (Treated Subjects in Studies CT-AMT-061-01 and CT-AMT-061-02)

	65 to 74 Years (Number of Older Subjects / Total No. of Subjects)	75 to 84 Years (Number of Older Subjects / Total No. of Subjects)	⩾ 85 Years (Number of Older Subjects / Total No. of Subjects)
Noncontrolled Studies (CT-AMT-061-01 and CT-AMT-061-02)	6/57	1/57	0/57
Study CT-AMT-061-01	0/3	0/3	0/3
Study CT-AMT-061-02	6/54	1/54	0/54

Of the 57 subjects treated with AMT-061, one subject was between 75 and 84 years of age and 6 subjects between 65 and 74 years of age.

2.6.5.4. In vitro biomarker test for patient selection for efficacy

The LUC-based AAV5-specific Neutralizing Antibody Assay has been adequately analytically validated, generally in line with the current ICH guidance for selectivity, specificity, precision, sensitivity, linearity, cross-reactivity, analytical cut point, carry-over, and sample stability.

According to the applicant, the assay is CE marked to Council Directive 98/79/EC, In-Vitro Diagnostic Medical Device Directive (IVDD).

The anti-AAV5 nAb assay used in Study CT-AMT-061-02 was planned to be utilised to screen patients previously diagnosed with haemophilia B to aid in the identification of patients with anti-AAV5 nAb levels who are eligible for treatment with etranacogene dezaparvovec. The cut-off titre proposed by the applicant to confirm treatment eligibility with Hemgenix was <1:700. This proposal is based on data from the pivotal trial where one subject with a nAb titre >1:3000 at baseline was found to be a non-responder to treatment with AMT-061, while all other twenty subjects who exhibited anti-AAV5 nAbs at baseline developed clinically relevant FIX activity levels and could terminate prophylaxis with exogenous FIX products. These subjects were found to have titres up to 1:678.2 at baseline, and apart from increased FIX activity they also reported a statistically significantly reduced annualised bleeding rate compared to the lead-in period using their ususal FIX prophylaxis. After etranacogene dezaparvovec administration, all 53 subjects developed detectable anti-AAV5 NAbs by Week 3 (median titre of 1:8,748; ULOQ titre = 1:8,748) which remained elevated through to Month 24 post dose.

However, in order to avoid a restriction of the indication with this arbitrary cut-off limit based on data from one patient only, the CAT requested to further investigate the effectiveness of Hemgenix in a post-authorisation efficacy study regardless of the preexisting anti-AAV5 nAb titre (PAES).

2.6.5.5. Analysis performed across trials (pooled analyses and meta-analysis)

Integrated Analysis of Durability of Response

An integrated exploratory analysis was performed on durability of response for etranacogene dezaparvovec for the treatment of haemophilia B. Two clinical studies were included: Study CT-AMT-061-01 and Study CT-AMT-061-02. The analysis used Month 6 post dose data as the baseline level,

while sensitivity analyses were performed using Month 3 post dose data as the baseline. The Month 6 baseline was chosen because a stable FIX activity level was observed by this time point, with most subjects not requiring prophylactic FIX replacement therapy or corticosteroid use for ALT elevation. The non-responder subject and the subject who received a partial dose were excluded from the durability analysis. Fifty-two out of 54 (96.3%) subjects did not need continuous prophylactic FIX replacement therapy from Day 21 through to Months 7 to 18, and the maximum duration of corticosteroid use for ALT elevation was 130 days in Study CT-AMT-061-02.

Additional supportive information on durability of response was provided by the proof-of-concept and FIH study of AMT-060 (the predecessor to etranacogene dezaparvovec), Study CT-AMT-060-01.

The primary objective of the integrated exploratory analysis was to characterise the durability of effect of etranacogene dezaparvovec in haemophilia B subjects. Study CT-AMT-061-01 and Study CT-AMT-061-02 were pooled for the integrated analysis.

The Durability of Effect Population included subjects who were enrolled, entered the Lead-in Period (the Lead-in Period was for Study CT-AMT-061-02 only), received a full dose of etranacogene dezaparvovec, and had at least 1 full year of assessments for FIX expression measured by FIX activity levels and FIX protein expression, and have baseline anti-AAV5 nab titre < 1:3,000. A total of 55 subjects (3 from Study CT-AMT-061-01 and 52 from Study CT-AMT-061-02) were part of the Durability of Effect Population. The analysis population for durability of response was the Durability of Effect Population, with supportive analyses using the AMT-060 Population. The AMT-060 Population included subjects in Study CT-AMT-060-01 who received 1 of 2 doses of AMT-060 and had at least 1 full year of assessments for FIX expression, measured by FIX activity levels and FIX protein expression. All 10 subjects from Study CT-AMT-060-01 were part of the AMT-060 Population. FIX activity (measured by one-stage [aPTT-based] assay) or FIX protein concentration values that were measured more than 5 half-lives after most recent FIX-replacement administration (uncontaminated values) were included in the durability analysis.

Percentage change and absolute change in uncontaminated FIX activity or FIX protein concentration from Month 6 (baseline) to each time point after Month 6 was evaluated using a linear mixed-effects repeated model, controlling for the fixed effects of Month 6 (baseline) FIX value and time point wherein the time point was treated as a categorical variable. In all analyses where convergence was achieved, a first-order autoregressive covariance structure provided the best fit as assessed by visual examination of the residuals along with goodness-of-fit test statistics output by the Statistical Analysis System (SAS) procedure MIXED.

Subgroup analysis was also conducted for durability in both FIX activity and FIX protein levels with an added covariate to define the subgroup, as well as adding an interaction term with visit. The following subgroups were evaluated: baseline anti-AAV5 nAb titre (positive / negative), ALT increase resulting in corticosteroid treatment (yes / no), and baseline FIX level (moderate / severe).

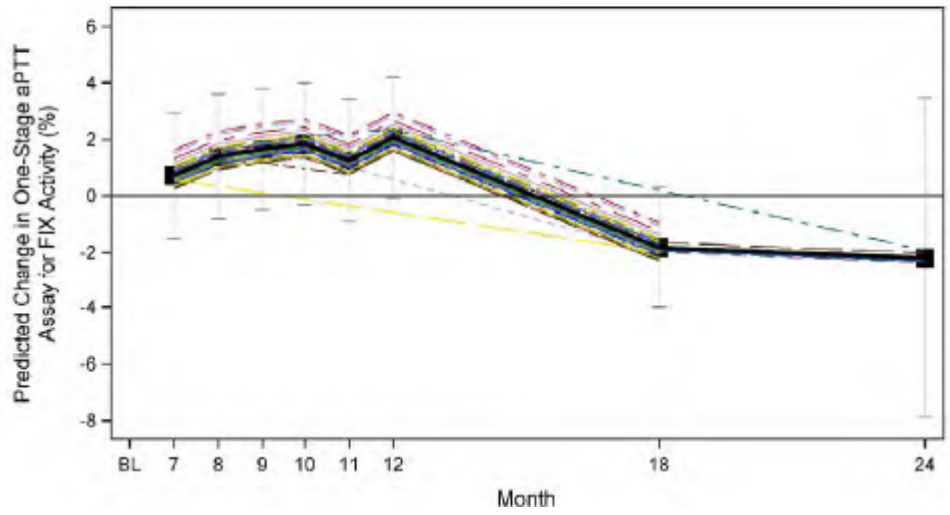
Durability of FIX Activity Levels

The analysis of durability of uncontaminated FIX activity levels, as measured by one-stage (aPTT-based) assay, for the pooled etranacogene dezaparvovec studies (Study CT-AMT-061-01 and Study CT-AMT-061-02) assessed as change from baseline and percent change from baseline showed that the least square (LS) mean FIX activity levels at Months 7, 8, 9, 10, 11, 12, and 18 were not statistically significantly different from the baseline at Month 6 (Figure 4).

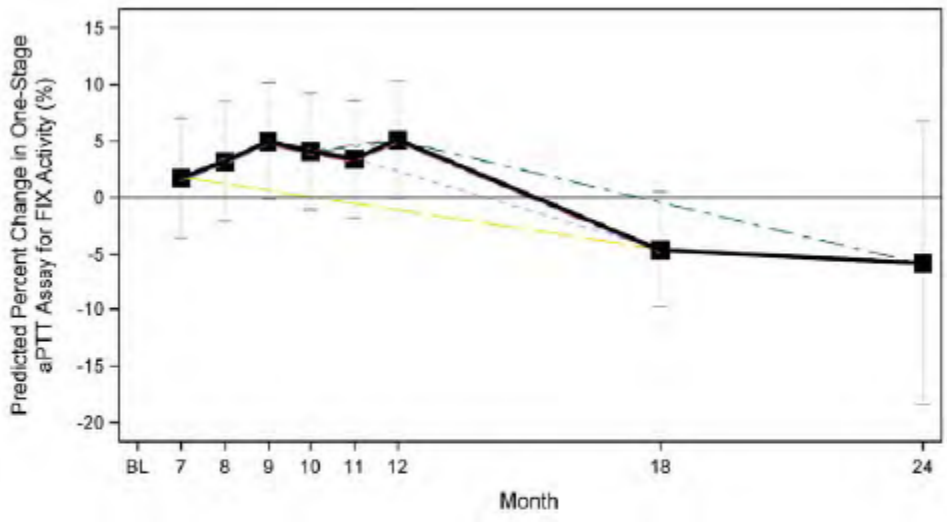
Similar to the FIX activity level changes for the time points shown above, FIX activity estimates at Month 24 showed a lack of difference from the Month 6 baseline. However, these were based on a limited sample size (n = 6 at Month 24) and data from additional subjects is needed to confirm this observation.

Figure 4. Least Squares Mean (95% CI) Change from Month 6 Baseline (A) and Percent Change from Month 6 Baseline (B) in FIX Activity Level (One-Stage [aPTT-based] Assay) after Etranacogene Dezaparvovec Administration (Durability of Effect Population)

A



B



aPTT = activated partial thromboplastin time; BL = baseline; CI = confidence interval; FIX = factor IX; LS = least squares; PK = pharmacokinetic.
BL is defined as the assessment done at the Month 6 visit if non-missing or, if missing, as the last assessment done before the Month 6 Visit. Observations collected within 5 half-lives after exogenous FIX exposure were considered contaminated and were excluded. Post Month 18 observations were excluded if fewer than 5 subjects had post Month 18 data. Colored lines are subject-level predicted values. Square markers (connected by a solid black line) are estimated values based on LS means (predicted population margins). Vertical dashed lines are 95% CIs for the LS means.

A sensitivity analysis was performed on the change in FIX activity level from baseline at Month 3, which showed a gradual increase over time of no more than 5% of normal mean activity up to Month 12 and no significant difference from Month 3 baseline at Months 18 and 24.

The durability analysis of change in FIX activity levels from baseline (Month 6) for AMT-060 at 2 dose levels (5×10^{12} gc/kg and 2×10^{13} gc/kg) was evaluated as supportive evidence (Durability and PK

Analysis). At both dose levels, the FIX activity levels at almost all time points up to 5 years were not statistically significantly different from the Month 6 baseline, with the exception of a marginal increase from baseline FIX activity at Months 12 and 24 at the high dose and at Month 54 at the low dose of 1.55%, 1.59%, and 4.53% of normal activity, respectively. A sensitivity analysis performed on the change in FIX activity level from baseline (Month 3) for the AMT-060 study showed similar durability up to 5 years from the Month 3 baseline.

FIX Activity Levels and FIX Protein Levels by Baseline anti-AAV5 nAb

The effect of preexisting anti-AAV5 nAbs on FIX activity levels after etranacogene dezaparvovec administration was evaluated by comparing FIX levels in subjects with and without preexisting anti-AAV5 nAbs in Study CT-AMT-061-02 (Baseline AAV5 nAb Titre Positive, N=20; PK Population).

At Month 6 to 18 post dose, subjects with and without preexisting anti-AAV5 nAbs had significant increases from baseline in FIX activity. No clinically meaningful differences in the mean FIX activity were observed between the 2 subgroups (up to 22% [at Month 18] lower FIX activity in subjects with preexisting anti-AAV5 nAbs relative to those without preexisting anti-AAV5 nAbs) at different time points post dose.

Although there was a trend of lower FIX activity in subjects with higher preexisting anti-AAV5 nAb titre, no clinically meaningful correlation between an individual's titre of preexisting anti-AAV5 nAbs with their FIX activity at 18 months post-treatment was identified up to an anti-AAV5 nAb titre of 1:3,212.3 (Pearson coefficient: -0.35; Spearman coefficient: -0.30; R2: 0.124.

FIX Activity Levels and FIX Protein Levels by Corticosteroid Use for ALT Elevation

After etranacogene dezaparvovec administration, 9 of 52 subjects in Study CT-AMT-061-02 had increased ALT treated with corticosteroids, while none of the 3 subjects in Study CT-AMT-061-01 had increased ALT treated with corticosteroids.

Mean FIX activity and FIX protein levels after etranacogene dezaparvovec administration at Months 6 to 18 were lower in subjects with ALT elevation treated with corticosteroids (9/55) (with the mean FIX activity was $\geq$ 15% of normal after Month 6), compared to the rest of the subjects in the studies (46/55) (Durability and PK Analysis). However, despite lower FIX activity in subjects using corticosteroids for ALT elevation, the durability of FIX activity response measured as change from baseline of FIX activity was sustained and generally not significantly different up to Month 18 from Month 6 baseline in both the subgroups with the exception of marginal change from baseline (Month 6) of -3.17% at Month 18 in subjects treated with corticosteroids for ALT elevation and < 3 % at Months 10 and 12 in the rest of the subjects (Table 14).

FIX Activity Levels and FIX Protein Levels by Baseline FIX Level

Based on FIX activity level at the time of diagnosis for subjects that received etranacogene dezaparvovec, 10/52 subjects in study CT-AMT-061-02 and 1/3 subjects in study CT-AMT-061-01 had moderately severe haemophilia ($\leq$ 2% of normal activity) while the rest of the subjects had severe haemophilia ($\leq$ 1% of normal activity).

Mean FIX activity and FIX protein levels after etranacogene dezaparvovec administration at 6 to 18 months were slightly lower in moderately severe haemophilia B subjects compared to those in severe haemophilia B subjects.

The provided analyses on the durability of endogenous FIX activity showed that, compared to month 6 as a baseline, there was a slight decline of FIX activity in the overall population at month 18. Data from study CT-AMT-060-01 also show a slow decline over a period of up to five years.

However, in all investigated subgroups (i.e. baseline anti-AAV5 nAb positive, treated with corticosteroids and suffering from moderate haemophilia), the decline was more pronounced, and this steeper decline occurred on top of lower FIX activity achieved at month 6. Therefore, it is likely that those subgroups will lose the benefit of endogenous FIX markedly earlier than patients without such influencing factors.

2.6.5.6. Supportive study

The open-label, uncontrolled study CT-AMT-060-01 was conducted with AAV5-hFIXco (AMT-060), the predecessor of etranacogene dezaparvovec, and is therefore considered supportive only.

No lead-in period was included, but a one-year observation period, during which historically reported bleeding episodes were documented. Two dose levels were administered with intra- and inter-cohort staggering intervals between IMP administrations for safety reasons. For the purpose of this study, this design is considered appropriate.

Five subjects each were enrolled in the lower dose Cohort 1 and higher dose Cohort 2. Demographic baseline data were generally balanced between cohorts, except the mean age, which was higher for subjects in Cohort 1 compared to Cohort 2 (60.2 years vs. 38.2 years). The explanation provided by the applicant can be followed.

The primary objective was to assess the 5-year safety profile of AMT-060, which was within the expected range. Most TEAE were mild or moderate in severity, one SAE of myelopathy was categorised as severe, not treatment-related, and resolved by study completion. Three SAEs (hepatic enzyme increased, pyrexia, ALT increased) were considered treatment-related, and the remaining SAEs (renal colic, calculus ureteric, myelopathy) were considered not (or unlikely) related by the Investigator, which can be agreed with based on the provided information. Three TEAE qualifying for special notification involving increased liver parameters were reported and treated with corticosteroids. Increased liver parameters are expected due to the mode of action of the IMP.

One death occurred outside the study period. The investigator assessed the relationship between AMT-060 and death as being unlikely related.

After treatment with AMT-060, the mean endogenous FIX activity levels in Cohort 1 and Cohort 2 ranged from 2.8% to 8.2% and 4.0% to 10.7% of normal based upon the one-stage (aPTT-based) FIX assay, respectively, and remained stable during the post-tapering period (i.e., after discontinuation of FIX prophylaxis post-AMT-060 administration) up to 5 years. Accordingly, the use of FIX replacement therapy and mean ABR were reduced after treatment with AMT-060 in both dose cohorts. Baseline as well as outcome data were more favourable in the higher dose Cohort 2, which could partially be attributed to the lower age.

No data are yet available for the corresponding long-term extension study CT-AMT-060-04, which is still ongoing. CSR is expected in September 2026 and will be reviewed during a later time point.

Overall, the safety and efficacy profile of AMT-060 would support a favourable benefit/risk balance of etranacogene dezaparvovec.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The clinical efficacy dataset of this marketing authorisation application is based on the results of two clinical trials investigating AMT-061 at a dose of 2×10^{13} gc/kg BW, i.e. Phase 2b trial CT-AMT-061-01

and pivotal Phase 3 trial CT-AMT-061-02. Limited supportive data are available from Phase 1 trial CT-AMT-060-01, which used a predecessor product, AMT-060, at two different dose levels.

CT-AMT-061-01 is a phase 2b open-label study in patients with severe or moderately severe haemophilia B. The primary objective of this study was to confirm that a single dose of 2×10^{13} gc/kg AMT-061 resulted in FIX activity levels of ≥5% at 6 weeks after dosing. Three subjects were treated. The primary efficacy endpoint was factor IX activity level at six weeks after dosing. Secondary endpoints were defined as endogenous factor IX activity level at Week 52 post AMT-061 dose, freedom of previous continuous routine prophylaxis, consumption of factor IX replacement therapy and annualised bleeding rate. Subjects will be followed for 5 years for efficacy and safety outcomes.

The applicant has not undertaken a proper dose finding study for etranacogene dezaparvovec. Only 3 patients were recruited into this first clinical study, based on the result derived from the predecessor product, AMT-060. However, these early results are encouraging and the efficacy results of the pivotal study are in-line with this dose finding study. Consequently, the issue of not performing a proper dose finding study was not further pursued.

The pivotal phase 3 Study CT-AMT-061-02 is a non-randomised, uncontrolled, open-label, single-arm trial of AMT-061. 54 subjects suffering from severe or moderately severe haemophilia B, who were treated with prior FIX prophylaxis were treated in the study. During the lead-in phase, which lasted a minimum of 26 weeks, subjects recorded their use of FIX replacement therapy and bleeding episodes in their dedicated e-diary in order to provide a baseline of bleeding event frequency and FIX consumption. Seventy-five subjects were screened, 67 subjects were enrolled and started the lead-in period. Fifty-four subjects received AMT-061 at a dose of 2×10^{13} gc/kg and provided data for efficacy and safety evaluation.

The inclusion and exclusion criteria select for generally healthy subjects with severe or moderately severe (FIX≤2) haemophilia, with a focus on excluding pre-existing significant hepatic disease. This precaution is endorsed as one of the most frequent expected short term adverse events is an increase in liver function parameters as the immunologic system tries to clear cells infected with the vector. In addition, patients with a history of FIX inhibitors were excluded from participation in the clinical investigation programme, which is adequately reflected in the SmPC.

The primary efficacy endpoint was defined as "Annualized bleeding rate comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable FIX expression (Months 6 to 18 post-treatment)". An increase of endogenous factor IX levels per se is considered desirable, however, the more relevant clinical outcome is the ensuing frequency of bleeding events. The prespecified run-in period of at least 26 weeks allows a comparison with the subjects' own ABR during replacement therapy with exogenous factor IX products and as such enhances the informative value of this non-randomised trial. Scientific advice was given several times during the clinical development of the product. However, the applicant has changed the primary efficacy endpoint during the ongoing study without solid clarification of this major change. While the final primary efficacy endpoint (ABR comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable FIX expression) is in principle acceptable, the applicant was asked to provide further justification of this change and possible influence of this change to the study outcome. The applicant has clearly and sufficiently justified why the change of the primary endpoint had no major impact on the interpretability or conclusion of the efficacy analysis.

Factor IX activity, consumption of external FIX and annual infusion rate were among the secondary efficacy outcomes, several PRO outcomes with regard to pain, activity and quality of life scores were also reported.

From a methodological perspective the overall approach is considered acceptable. The trial design was discussed and agreed upon during PRIME scientific advice interactions and is in principle endorsed, i.e. no comparator arm and ABR (comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable FIX expression) as a primary efficacy endpoint. Thus patients serve as their own controls, i.e. an intra-patient comparison instead of a randomised comparison is performed. However, the method for the interval estimation of the annualised bleeding rate ratio is not considered optimal.

The applicant was asked to explain why the data collection method for the primary endpoint was changed in the middle of the trial and in the middle of the efficacy period for the primary analysis. Furthermore, the applicant was asked to elaborate on the data collection method during the lead-in study. The applicant was requested to provide convincing rationale that the bleed data is truly comparable between the three study periods (lead-in, up to Week 52 and after Week 52). Lastly, the applicant was requested to explain in detail how it was ensured that days with no bleeds recorded are truly days with no bleeds and not missing data. With the responses to the D120 LoQ, the applicant provided sufficient clarification on the methods of data collection for the primary endpoint. The eDiary collection is considered to cover the most important periods (i.e. the Lead-in and months 7 to 12 postdose). The applicant was advised to ensure continued data collection.

No pre-planned analyses to assess the compliance to complete the bleed questionnaire have been identified and no results are found in the CSR. A comprehensive assessment of compliance was requested, including the lead-in and actual study and electronic and paper diaries. Importantly, days when no bleeds were recorded should be distinguishable from days when the patient was not compliant. The rules given to the patients on how and when to complete the diaries should be provided. With the responses to the D120 LoQ, a comprehensive assessment of compliance was provided, as requested and for most patients the compliance was good in regard to reporting.

Interim analysis: The rationale for the 6-month and 12-month interim analyses for endogenous factor IX activity is not followed. It is understood that the study team had access to the results which is not acceptable though this is an open-label study. Access to the aggregated results should not be available until the primary analysis. The applicant was requested to confirm which parties had access to the results of the interim analyses, what was externally communicated and explain why they were conducted. It is notable that both the protocol and the SAP have been amended after the performed interim analyses. The requested details were provided by the applicant in regard to the conduct of the interim analysis (i.e. timing, data access, etc.). The results were communicated to the scientific community in the context of various conferences, beginning in 2019, which is considered understandable with regards to the novelty of etranacogene dezaparvovec gene therapy.

In order for an intra-patient comparison to provide an unbiased estimate, all patients should have moved from the lead-in period to the active part of the study. However, 13 out 67 patients discontinued from the lead-in period. Only high-level information with regard to number of exclusion criterion is provided. Due to that, the applicant was asked to provide exact details from all patients excluded during the screening and lead-in period, preferably in a tabulated format. Furthermore, the applicant was asked to justify the generalizability of the efficacy results considering the already low number of patients in this single pivotal study (n= 54) together with the fact that 19% of the patients in the lead-in period did not continue into the actual study. The details on screen failures were provided and the presented reasons are considered adequate. In addition, the applicant has provided the requested rationale for generalizability of the efficacy results, which imply that the bleeding tendencies before the treatment were comparable.

The applicant has made several, important amendments to this single pivotal study. From the data provided it is difficult to outline how many patients were treated before each of the performed

Amendment. The applicant was asked to provide this information. The applicant was also asked to clarify how reliable the results of the changed primary endpoint are (ABR, which is considered to be subjective at risk of possible bias), due to between- and within-subject variation to reporting bleeds to the ePRO, paper diary or to treatment centre. In their response, the applicant provided the requested information on the patients treated before each protocol amendment. All patients were treated before the change of the primary endpoint. The applicant has also elaborated on the reliability of the results due to the change in primary endpoint.

Overall, the applicant has provided all the information requested and all methodological issues are considered resolved.

Efficacy data and additional analyses

Efficacy data for the proposed dose of 2×10^{13} gc/kg are available from the **3** subjects in trial **CT-**AMT-061-01 and from 54 subjects from trial CT-AMT-061-02. Supportive data with the predecessor product AMT-060 are available from a phase 1 trial in 2 cohorts of 5 subjects each from study CT-AMT-060-01.

In the phase 2b study CT-AMT-061-01, mean FIX activity level at Week 6, the time of the primary endpoint read-out, was 30.6 % measured by the one-stage assay. Individual FIX activity levels achieved by each subject at Week 6 were 23.9%, 30.0%, and 37.8%. At Week 52, the mean FIX activity level was 40.8% measured by the one-stage assay. Individual FIX activity levels achieved by each subject at Week 52 were 31.3%, 40.8%, and 50.2%. At Month 36, uncontaminated samples were available for 2 subjects and demonstrated that FIX activity levels continued to be elevated, at 32.3% and 41.5%, respectively.

A discrepancy is noted between measuring FIX activity with the one-stage or the chromogenic assay. The chromogenic assay returns values approximately half of the values observed with the one-stage assay. A warning statement was introduced to section 4.4 of the SmPC to alert the treating physician to the fact that the chromogenic assay returns lower FIX activity values than the one-stage assay, with a mean ratio of FIX activity by chromogenic assay to one-stage (aPTT-based) assay from 0.408 to 0.547.

The average ABR for the 3 subjects was 0.27 over the period of 2.5 years of follow-up. The ABRs for spontaneous and traumatic bleeding episodes over 2.5 years were both 0.14. The average ABR for the 3 subjects was 0.22 over the period of 3 years (36 months) of follow-up. The ABRs for spontaneous and traumatic bleeding episodes over 3 years (36 months) were both 0.11. There were no bleeding episodes between 2.5 and 3 years of follow-up (both bleeding episodes occurred in the first 18 months post-AMT-061 administration). These ABR values are low, but as this trial had no run-in phase specified in the protocol, a comparison to meaningful pre-treatment data is not possible.

In the pivotal trial CT-AMT-061-02, a significant reduction of unadjusted mean ABR could be shown comparing the lead-in period ABR of 4.11 to the post-treatment ABR of 1.08 recorded during months 7 to 18. The prespecified NI analysis encompassing a comparison of ABR between the lead-in and post-treatment period estimated from a negative binomial regression model was significant and non-inferiority to FIX prophylaxis could be declared. In addition, the secondary outcome of superiority over FIX prophylaxis could also be shown. 20.4% of subjects reported joint bleeding episodes post-treatment, compared to 59.3% of during lead-in. The number of subjects who did not experience any bleeding event more than doubled during month 7-18 [34/54 (63.0%)] compared to baseline [14/54 (25.9%)]. Sensitivity analyses are in line with observed ABR results. In the subgroup analysis, ABR was significantly reduced during months 7 to 18 after AMT-061 treatment compared to the lead-in period for most of the subgroups analysed, except subjects with a positive anti-AAV5 nAb titre at baseline (n = 21) most likely due to one patient with high pre-dose anti-AAV5 nAb titre >3000 (3212).

With the responses to the D120 LoQ, an ABR analysis for months 7-24 after treatment was provided (not adjusted for multiplicity). The unadjusted ABR was 0.99, with the adjusted ABR 1.51 (0.83, 4.76). 27 (50.0%) of subjects reported no bleeding episode from month 7-24. 27.8% of subjects reported joint bleeds between month 7-24.

FIX activity levels showed clinically relevant values at month 6 (mean 38.95; median 37.30), continued to increase until month 12 (mean 41.48; median 39.90) and then declined slightly until month 18 (mean 36.90; median 35.55) and remained steady at month 24 (mean 36.66; median 33.85). No subject recorded values >150%. External factor IX consumption as well as external FIX infusion rate in the post-treatment period declined to approximately 3% of the value observed during the lead-in period. Fifty-two of 54 subjects remained free from FIX replacement therapy during the follow-up period of 18 months. One of the two subjects who had to return to FIX replacement therapy received only about 10% of the intended dose of AMT-061 due to hypersensitivity and the second had a high anti-AAV5 nAb titre at baseline and did not respond to treatment with Hemgenix.

All investigated subgroups showed an improvement with regard to ABR and FIX activity level in the post-treatment period compared to the lead-in period. The subgroup of subjects demonstrating appreciably lower FIX activity levels compared to the other subgroups was comprised of those patients who experienced an ALT elevation post AMT-061 treatment and especially those patients who received corticosteroids as a consequence. The highest FIX activity during months 7-18 in a patient treated with corticosteroids was ~30% and the lowest only 4.5%.

The ideal outcome of the therapy in the long-term, in addition to reduction of ABR, would of course be freedom of previous intravenous FIX substitution therapy. Importantly, 34/53 (64.2%) subjects whose baseline anti-AAV5 nAb titre was <3000 had 0 bleeding episodes during the Month 7 to 18 post-treatment period and 27/53 (50.9) subjects had zero bleeds during Month 7-24 period.

Subjects were enrolled into the pivotal study irrespective of their pre-existing anti-AAV nAb titre. Twenty subjects were found to have titres up to 1:678.2 at baseline, and 33 subjects were negative. While overall a numerically lower mean Factor IX activity was observed in patients with pre-existing neutralising anti-AAV5 antibodies, no clinically meaningful correlation was identified between patients' pre-existing anti-AAV5 antibody titre and their factor IX activity at 18 months post-dose. In 1 patient with a titre of 1:3212 for pre-existing anti-AAV5 antibodies at screening, no response to etranacogene dezaparvovec treatment was observed, with no factor IX expression and activity.

In consequence, the applicant proposed a cut-off titre of <1:700 to be introduced into the product information which was intended to determine treatment eligibility with Hemgenix. However, in order to avoid a restriction of the indication with this arbitrary cut-off limit based on data from one patient only, the CAT was of the view that the investigation of the effectiveness of Hemgenix in a post-authorisation efficacy study regardless of the preexisting anti-AAV5 nAb titre (PAES) is the preferred option.

Minimal or no improvements could be detected in patient reported outcome scales, several of which were investigated during the clinical trial (e.g. WPAI, BPI, HAL and EQ-5D-5L).

The use of corticosteroids for elevation of transaminases was prespecified in the protocol and is adequately reflected in section 4.4 of the SmPC. Thirteen out of 53 (24.5%) subjects who received a full dose of AMT-061 experienced ALT elevation and 9 (16.9%) subjects were treated with corticosteroids. The duration of corticosteroid use for elevated transaminases ranged from 51 to 130 days.

In the supportive study CT-AMT-060-01 efficacy was demonstrated by continuously increased endogenous FIX activity levels throughout the study duration up to 5 years. Accordingly, the use of FIX replacement therapy and mean ABR were reduced after treatment with AMT-060 in both dose cohorts.

Additional efficacy data needed in the context of a conditional MA

The final clinical study report including 5 years follow-up of Study CT-AMT-061-01 should be submitted no later than June 2024 and is subject to a specific obligation laid down in the MA (SOB-1).

The final clinical study report including 5 years follow-up of the pivotal Study CT-AMT-061-02 with 54 subjects should be submitted no later than October 2025 and is subject to a specific obligation laid down in the MA (SOB-2).

A one year follow-up interim analysis report after the first 50 subjects are enrolled in Study CSL222_4001 should be provided no later than December 2026 (SOB-3).

2.6.7. Conclusions on the clinical efficacy

The submitted clinical efficacy data show a statistically significant and clinically relevant improvement of ABR in the post-treatment period (months 7-24) compared to the lead-in period of at least 6 months, during which subjects were receiving prophylactic FIX replacement.

Endogenous FIX activity achieved clinically relevant levels in the majority (52/54) of subjects, with no subject showing supraphysiologic FIX activity. Use of exogenous FIX as well as FIX infusion rate fell to approximately 3% of values reported during lead-in.

In order to further elucidate the durability of the response, the applicant was asked to submit the 2-year efficacy data for the primary and all secondary efficacy endpoints and to develop a quantitative pharmacokinetic model that estimates the durability of FIX activity in the general clinical trial population, and also in relevant subgroups. Updated efficacy data from 24 months of follow-up from pivotal trial AMT-061-02 and 36 months of follow-up from trial AMT-061-01 continue to show satisfactory outcomes with regard to clinically relevant FIX activity and a sustained low ABR. The durability of the therapeutic effect has been shown to be stable up until 24 months of follow-up in the pivotal trial.

The initially proposed wording of the indication with regard to the threshold for baseline anti AAV5 nAb was not accepted by CAT. In order to avoid an arbitrary cut-off limit based on data from one patient and taking into account that patients were enrolled into the pivotal study irrespective of their baseline nAb titre, this restriction on the indication was removed from the wording of 4.1.

However, while the two years of follow-up provided show that the expression of FIX activity appears to be stable over this duration, the long-term durability of the treatment effect and long-term safety are still unknown factors. In addition, since uncertainties regarding the impact of neutralizing anti-AAV capsid antibodies on efficacy and safety cannot be comprehensively characterised based on available data, a full MA as sought by the applicant was not considered acceptable.

Due to the limitations of the provided dataset, the applicant agreed to request a conditional marketing authorisation. The prerequisites for a CMA according to Commission Regulation (EC) No 507/2006 are considered as fulfilled.

The CAT considers the following measures necessary to address the missing efficacy data in the context of a conditional MA:

- In order to confirm the efficacy and safety of etranacogene dezaparvovec in adult patients with severe and moderately severe Haemophilia B (congenital Factor IX deficiency) without a history of Factor IX inhibitors, the MAH should submit the final results including 5 years follow-up of the pivotal Study CT-AMT-061-01.
- In order to confirm the efficacy and safety of etranacogene dezaparvovec in adult patients with

severe and moderately severe Haemophilia B (congenital Factor IX deficiency) without a history of Factor IX inhibitors, the MAH should submit the final results (5 years of data) of pivotal Study CT-AMT-061-02 with 54 subjects.

- In order to confirm the efficacy and safety of etranacogene dezaparvovec in adult patients with severe and moderately severe Haemophilia B (congenital Factor IX deficiency) without a history of Factor IX inhibitors, irrespective of baseline anti-AAV5 neutralising antibody titre, the MAH should submit a 1-year follow-up interim analysis report after the first 50 subjects are enrolled in Study CSL222_4001.

The CAT considers the following measures necessary to address issues related to efficacy:

- In order to further characterise the long-term efficacy and safety of etranacogene dezaparvovec in adult patients with severe and moderately severe Haemophilia B (congenital Factor IX deficiency) without a history of Factor IX inhibitors, the MAH should submit the final analysis report of a study from a registry, according to an agreed protocol.

The CHMP endorses the CAT conclusion on clinical efficacy as described above.

2.6.8. Clinical safety

Two clinical studies provide safety data for etranacogene dezaparvovec (AMT-061, human FIXco-Padua) from in total 57 exposed adult subjects with severe or moderately severe haemophilia B, with and without pre-existing nAbs to the AAV5 capsid.

- **Study CT-AMT-061-01** is an ongoing, 5-year, phase 2b, open-label, single-dose, single-arm, multicenter study (n=3, 3-year post-dose, database lock: 28 February 2022).
- **Study CT-AMT-061-02** (HOPE-B [Health Outcomes with Padua gene; Evaluation in Hemophilia B]) is an ongoing, pivotal, phase 3, open-label, single-dose, single-arm, multinational study, which included a ≥ 6-month Lead-in Period with standard of care continuous FIX prophylaxis (n=54, 2-year post-dose data, database lock: 21 April 2022).

Following an initial data submission, the applicant provided a safety data update upon request, including additional 6 months of data (until the above-described cut-off dates).

Nearly all subjects were exposed to a single dose of 2×10^{13} gc/kg AMT-061, except for one participant who received a partial dose (~10%).

The safety endpoints in both studies are: AEs; Anti-AAV5 antibodies (total [IgM and IgG], nAb); AAV5 capsid-specific T-cells; Anti-FIX antibodies; FIX inhibitors and recovery; Haematology and serum chemistry parameters; ALT and AST levels, and corticosteroid use for ALT and AST increases; Vector DNA in blood and semen; Inflammatory markers: interleukin (IL)-1β, IL-2, IL-6, interferon gamma (IFNγ), monocyte chemoattractant protein-1 (MCP-1); AFP; Vital signs (including abdominal ultrasound).

Supportive safety data are available from Study CT-AMT-060-01, a Phase I/II open label, uncontrolled, single-dose, dose-ascending, multi-center study investigating two dose levels (5×10^{12} gc/kg, 2×10^{13} gc/kg, n=5 per dose) of AMT-060 (FIX wild type). The final CSR is available (5-year data).

2.6.8.1. Patient exposure

Table 17. Completed and Ongoing Clinical Studies with Data Included in the Safety Summary

Study ID No. of Centers / Location	Database Lock / Data Duration	Endpoints of the Study	Phase Study Design	Study Population	Test Product Dose Regimen	Number of Subjects Planned and Enrolled / Treated	Sex Age Range Race Distribution
CT-AMT-061-01 4 sites (1 subject switched sites)	28 February 2022 / 3-year data (Ongoing)	Primary Endpoint: FIX activity level at Week 6 postdose	Phase 2b, open-label, single-dose, multicenter, study investigating the safety, tolerability, and efficacy of etranacogene dezaparvovec	Adult subjects with severe or moderately severe hemophilia B	Etranacogene dezaparvovec Single 2×10^{13} gc/kg IV dose	3 subjects planned, enrolled, and treated	Sex: Males Age range: 43 to 50 years [redacted] Black: 66.7% White: 33.3%
CT-AMT-061-02 33 sites	21 April 2022 / 2-year (Ongoing)	Primary Endpoint: ABR comparison between etranacogene dezaparvovec and (standard of care hFIX) prophylaxis for noninferiority between the Lead-in Period and the 52 weeks following stable hFIX expression (Months 7 to 18 postdose)	Phase 3, open-label, single-dose, multicenter, multinational study investigating the safety, tolerability, and efficacy of etranacogene dezaparvovec	Adult subjects with severe or moderately severe hemophilia B	Etranacogene dezaparvovec Single 2×10^{13} gc/kg IV dose	At least 50 subjects planned 67 subjects enrolled 54 subjects treated; 53 subjects received planned dose; 1 subject received partial dose (approximately 10%)	Sex: Males Age range: 19 to 75 years [redacted] White: 74.1% Other: 11.1% Missing: 9.3% Asian: 3.7% Black: 1.9%

ABR = annualized bleeding rate; gc/kg = genome copies per kilogram; FIX = Factor IX; hFIX = human Factor IX; IV = intravenous.

All subjects were male, which is acceptable as haemophilia B is an X-linked recessive condition and occurs primarily in men. The majority identified as White (n=41, 78.8%). The **mean (SD) age** at baseline of the treated subjects was **41.7 years (15.42)** and the median age was 37 years. The mean BMI was 27.06 kg/m^2, ranging from 21.2 to 51.0 kg/m^2. The majority of subjects was in the age group between 18-49 years (71.9%, n=41), 15.8% (n=9) were between 50-64 years, and 12.3% (n=7) were between 65-75 years of age at baseline.

At the time of their diagnosis, 47/57 (82.5%) subjects had severe haemophilia B. The remaining subjects (17.5%, n=10) were diagnosed with moderately severe haemophilia B. Five (8.8%) participants were HIV positive, 9/57 (15.8%) subjects reported a history of hepatitis B, and 34/57 (59.7%) subjects reported a history of hepatitis C. At baseline, all participants were non-reactive to HBsAg and nine (15.8%) were reactive to HBeAg.

A considerable number of subjects were seropositive for anti-AAV5 nAbs before treatment with AMT-061 (n= 24, 42.1%).

Table 18. Investigational Product Exposure Duration (ISS Safety Population)

	Study CT-AMT-061-01 (N = 3)		Study CT-AMT-061-02 (N = 54)		Total Etranacogene Dezaparvovec (N = 57)	
	n	Person-months[a]	n	Person-months[a]	n	Person-months[a]
Exposure Duration[b,c]						
< 1 month	0	0	0	0	0	0
1 to < 3 months	0	0	0	0	0	0
3 to < 6 months	0	0	0	0	0	0
6 to < 12 months	0	0	0	0	0	0
12 to < 18 months	0	0	1	15.2	1	15.2
18 to < 24 months	0	0	2	47.5	2	47.5
24 to < 36 months	0	0	50	1329.8	50	1329.8
36 to < 48 months	3	118.0	1	37.0	4	154.9
48 to < 60 months	0	0	0	0	0	0
≥ 60 months	0	0	0	0	0	0
Total person-months[a]	3	118.0	54	1429.5	57	1547.5

[a] Person-months is the total number of months contributed to each exposure duration interval.
[b] Exposure duration is defined as time on study from treatment date to the minimum of End-of-study Visit date, early termination date, or data cutoff date.

In total, 57 subjects were followed-up for 1547.5 person-months post dose. Fifty-four participants have a duration of exposure >24 months. One subject died on study day 464.

2.6.8.2. Adverse events

Lead-in Period

Table 19. Overall Summary of Adverse Events in ≥ 2 Percent by Preferred Term - Study CT-AMT-061-02 (Study Safety Population)

	Lead-in Period (Including Lead-in Discontinuers) (N = 67)		Lead-in Period (Excluding Lead-in Discontinuers) (N = 54)	
Preferred Term[a]	n (%)	Events	n (%)	Events
At least 1 AE	42 (62.7)	103	37 (68.5)	87
Nasopharyngitis	8 (11.9)	8	8 (14.8)	8
Arthralgia	5 (7.5)	5	4 (7.4)	4
Oropharyngeal Pain	3 (4.5)	3	2 (3.7)	2
Cystitis	2 (3.0)	2	2 (3.7)	2
Upper Respiratory Infection	2 (3.0)	2	2 (3.7)	2
Joint Swelling	2 (3.0)	2	2 (3.7)	2
Nausea	2 (3.0)	2	2 (3.7)	2
Toothache	2 (3.0)	2	2 (3.7)	2
Dry Skin	2 (3.0)	2	2 (3.7)	2
Iron Deficiency Anaemia	2 (3.0)	2	2 (3.7)	2
Insomnia	2 (3.0)	3	2 (3.7)	3
Rhinitis	2 (3.0)	2	1 (1.9)	1
Haemarthrosis	2 (3.0)	2	1 (1.9)	1
Pain	2 (3.0)	2	1 (1.9)	1
Anaemia	2 (3.0)	2	1 (1.9)	1

AE = adverse event; SOC = system organ class.
[a] Preferred terms are presented by descending frequency in all subjects and secondarily grouped by SOC.

During the lead-in period (≥ 6 months), the subjects received standard of care continuous routine factor IX prophylaxis. Of the 54 subjects who finished the lead-in period (and received treatment with

AMT-061), 37 (68.5%) reported 87 AEs, with nasopharyngitis (n=8, 14.8%) and arthralgia (n=4, 7.4%) as the most frequently reported AEs. Overall, it appears that many AEs could have been caused by background disease (e.g., due to respiratory tract infection) or other pre-existing conditions (haemarthrosis).

Treatment-emergent Adverse Events (TEAEs)

Table 20. Incidence and Number of Treatment-emergent Adverse Events (ISS Safety Population)

	Study CT-AMT-061-01 (N = 3)		Study CT-AMT-061-02 (N = 54)		Total Etranacogene Dezaparvovec (N = 57)	
	n (%)	Events	n (%)	Events	n (%)	Events
Any TEAE	3 (100)	56	54 (100)	557	57 (100)	613
TEAE by Severity[a]						
Mild	2 (66.7)	36	54 (100)	424	56 (98.2)	460
Moderate	2 (66.7)	20	37 (68.5)	115	39 (68.4)	135
Severe	0	0	11 (20.4)	18	11 (19.3)	18
Serious TEAE (SAE)	1 (33.3)	1	14 (25.9)	17	15 (26.3)	18
Treatment-related TEAE[b]	1 (33.3)	2	38 (70.4)	93	39 (68.4)	95
Serious and Treatment-related TEAE	0	0	0	0	0	0
TEAE Leading to Study Treatment Discontinuation[c]	0	0	1 (1.9)	1	1 (1.8)	1
AEQSN	0	0	12 (22.2)	19	12 (21.1)	19
Fatal TEAE (death)	0	0	1 (1.9)	1	1 (1.8)	1

AEQSN = Adverse Event Qualifying for Special Notification; SAE = serious adverse event; TEAE = treatment-emergent adverse event.
[a] For each severity level, subjects are counted if they had any TEAE with the given severity level.
[b] Related or possibly related to study treatment.
[c] Defined as receiving a partial dose.
Notes:
1. TEAEs are adverse events with onset date on or after the date of etranacogene dezaparvovec administration.
2. AEQSN: Subject met 1 or more of the following criteria: 1) related to the etranacogene dezaparvovec administration procedure, 2) suspected or confirmed case of opportunistic or serious infection that was related to etranacogene dezaparvovec, 3) unexpected reaction related to product failure, 4) related to mandatory concomitant medication, 5) related to medical devices that formed part of the product or were used for application of the product, and 6) new or recurrent cancer.

The incidence and number of adverse events is clearly higher after treatment than during the lead-in period. This may partly be due to longer time of follow-up after the treatment compared to the lead-in period. Also, some AEs may occur shortly after treatment, which increases the amount of AEs in the after-treatment period compared to the lead-in period. The applicant was requested to show and discuss, how the AE profile of treated subjects changes during the follow-up period, and to show a comparison of 1) AEs of the lead-in period, 2) 0-1 month, 3) AEs of the 0-6 months follow-up period after treatment and 4) AEs of the 6-18 months follow-up period after treatment.

The applicant presented the comparison of the four time frames in tabulated format as requested (table not shown here). During the Lead-in period (excluding lead-in discontinuers), 68.5% experienced an AE, 85.2% had AEs in the first month postdose and 98.1% had AEs during months 0 to 6 postdose. Thereafter, the incidence of AEs reduced to 74.1% from months 7 to 18 postdose.

The higher incidence of AEs during the first 6 months postdose compared with the Lead-in Period and the period from 7 to 18 months post-dose can be explained by AEs which reflect an immune response against the vector. These AEs include infusion related reactions, flu-like symptoms (including headache, fatigue, and influenza like illness), C-reactive protein increased, blood creatine phosphokinase increased, and liver enzyme elevations (including ALT Increased, and AST increased). No other pattern of TEAEs related to etranacogene dezaparvovec was identified.

Adverse events expected in this haemophilia B population (such as Hemarthrosis, Arthralgia, or Gastrointestinal Haemorrhage, and other events like seasonal infections or comorbidities due to the

subject's medical history or age) occurred at a comparable frequency during the 4 periods (Lead-in Period, Month 0 to 1, Months 0 to 6, and Months 7 to 18).

Common TEAEs

The most commonly reported TEAEs by PT, irrespective of investigator causality assessment, were Arthralgia (36.8%), Headache (31.6%), Nasopharyngitis (26.3%), Fatigue (24.6%), and ALT Increased (21.1%), as shown below. No trends were discernible in TEAEs based on age, race, ethnicity, or BMI.

Table 21: Incidence and Number of Treatment-emergent Adverse Events by Preferred Term in ≥ 5% of Subjects (ISS Safety Population)

	Study CT-AMT-061-01 (N = 3)		Study CT-AMT-061-02 (N = 54)		Total Etranacogene Dezaparvovec (N = 57)	
Preferred Term[a]	**n (%)**	**Events**	**n (%)**	**Events**	**n (%)**	**Events**
Any TEAE	3 (100)	56	54 (100)	557	57 (100)	613
Arthralgia	2 (66.7)	3	19 (35.2)	34	21 (36.8)	37
Headache	2 (66.7)	4	16 (29.6)	31	18 (31.6)	35
Nasopharyngitis	0	0	15 (27.8)	20	15 (26.3)	20
Fatigue	0	0	14 (25.9)	17	14 (24.6)	17
Alanine Aminotransferase Increased	1 (33.3)	2	11 (20.4)	12	12 (21.1)	14
Back Pain	2 (66.7)	2	9 (16.7)	12	11 (19.3)	14
COVID-19	0	0	10 (18.5)	10	10 (17.5)	10
Pain in Extremity	0	0	9 (16.7)	10	9 (15.8)	10
Aspartate Aminotransferase Increased	1 (33.3)	1	8 (14.8)	9	9 (15.8)	10
Blood Creatine Phosphokinase Increased	1 (33.3)	1	8 (14.8)	11	9 (15.8)	12
Influenza-like Illness	0	0	7 (13.0)	12	7 (12.3)	12
Oropharyngeal Pain	0	0	7 (13.0)	7	7 (12.3)	7
Toothache	0	0	7 (13.0)	11	7 (12.3)	11
Hypertension	1 (33.3)	1	6 (11.1)	6	7 (12.3)	7
Cough	0	0	6 (11.1)	6	6 (10.5)	6
Diarrhoea	0	0	6 (11.1)	6	6 (10.5)	6
Nausea	0	0	6 (11.1)	6	6 (10.5)	6
Ligament Sprain	0	0	5 (9.3)	5	5 (8.8)	5
Malaise	0	0	5 (9.3)	7	5 (8.8)	7
C-Reactive Protein Increased	1 (33.3)	1	4 (7.4)	4	5 (8.8)	5
Chest Pain	1 (33.3)	3	4 (7.4)	4	5 (8.8)	7
Dizziness	1 (33.3)	2	4 (7.4)	4	5 (8.8)	6
Pain	1 (33.3)	6	4 (7.4)	4	5 (8.8)	10
Anaemia	0	0	4 (7.4)	4	4 (7.0)	4
Haemorrhoids	0	0	4 (7.4)	4	4 (7.0)	4

Hepatic Steatosis	0	0	4 (7.4)	4	4 (7.0)	4
Myalgia	0	0	4 (7.4)	4	4 (7.0)	4
Pyrexia	0	0	4 (7.4)	5	4 (7.0)	5
Rhinorrhoea	0	0	4 (7.4)	4	4 (7.0)	4
Influenza	1 (33.3)	1	3 (5.6)	3	4 (7.0)	4
Upper Respiratory Tract Infection	1 (33.3)	2	3 (5.6)	3	4 (7.0)	5
Abdominal Pain Upper	0	0	3 (5.6)	4	3 (5.3)	4
Arthritis	0	0	3 (5.6)	3	3 (5.3)	3
Chills	0	0	3 (5.6)	3	3 (5.3)	3
Contusion	0	0	3 (5.6)	3	3 (5.3)	3
Cystitis	0	0	3 (5.6)	4	3 (5.3)	4
Infusion Related Reaction	0	0	3 (5.6)	3	3 (5.3)	3
Iron Deficiency Anaemia	0	0	3 (5.6)	3	3 (5.3)	3
Limb Injury	0	0	3 (5.6)	4	3 (5.3)	4
Musculoskeletal Chest Pain	0	0	3 (5.6)	3	3 (5.3)	3
Vitamin D Deficiency	0	0	3 (5.6)	3	3 (5.3)	3
Joint Swelling	1 (33.3)	1	2 (3.7)	2	3 (5.3)	3
Paraesthesia	1 (33.3)	2	2 (3.7)	2	3 (5.3)	4
Tachycardia	1 (33.3)	1	2 (3.7)	2	3 (5.3)	3

COVID-19 = Coronavirus disease 2019; TEAE = treatment-emergent adverse event;

[a] Preferred terms were sorted by descending incidence among subjects in combined studies, and by descending incidence in CT-AMT-061-02, then CT-AMT-061-01 for ties.

TEAEs were adverse events with onset date on or after the date of etranacogene dezaparvovec administration.

Treatment-emergent Adverse Events Related to Study Treatment

In the ISS Safety Population, 68.4% of subjects experienced treatment-related TEAEs. Most common treatment-related TEAEs by PT were headache (9 [15.8%]), ALT Increased (9 [15.8%]), influenza-like Illness (7 [12.3%]), and AST increased (5 [8.8%]) as shown in the Table below. No trends were noted in treatment-related TEAEs based on age, race, ethnicity, or BMI.

Table 22: Incidence and Number of Treatment-emergent Adverse Events Related to Study Treatment by Preferred Term (ISS Safety Population)

	Study CT-AMT-061-01 (N = 3)		Study CT-AMT-061-02 (N = 54)		Total Etranacogene Dezaparvovec (N = 57)	
Preferred Term	**n (%)**	**Events**	**n (%)**	**Events**	**n (%)**	**Events**
Any TEAE Related to Study Treatment[a]	1 (33.3)	2	38 (70.4)	93	39 (68.4)	95
Alanine Aminotransferase Increased	0	0	9 (16.7)	10	9 (15.8)	10
Headache	1 (33.3)	1	8 (14.8)	9	9 (15.8)	10
Influenza-like Illness	0	0	7 (13.0)	8	7 (12.3)	8
Aspartate Aminotransferase Increased	0	0	5 (9.3)	6	5 (8.8)	6

Blood Creatine Phosphokinase Increased	0	0	4 (7.4)	6	4 (7.0)	6
Dizziness	0	0	4 (7.4)	4	4 (7.0)	4
Fatigue	0	0	4 (7.4)	4	4 (7.0)	4
Nausea	0	0	4 (7.4)	4	4 (7.0)	4
Arthralgia	0	0	3 (5.6)	3	3 (5.3)	3
Infusion Related Reaction	0	0	3 (5.6)	3	3 (5.3)	3
C-reactive Protein Increased	1 (33.3)	1	2 (3.7)	2	3 (5.3)	3
Abdominal Discomfort	0	0	2 (3.7)	2	2 (3.5)	2
Chills	0	0	2 (3.7)	2	2 (3.5)	2
Diarrhoea	0	0	2 (3.7)	2	2 (3.5)	2
Malaise	0	0	2 (3.7)	3	2 (3.5)	3
Pain	0	0	2 (3.7)	2	2 (3.5)	2
Abdominal Pain Upper	0	0	1 (1.9)	1	1 (1.8)	1
Anaemia	0	0	1 (1.9)	1	1 (1.8)	1
Back Pain	0	0	1 (1.9)	1	1 (1.8)	1
Blood Bilirubin Increased	0	0	1 (1.9)	1	1 (1.8)	1
Chest Discomfort	0	0	1 (1.9)	1	1 (1.8)	1
Coagulation Factor IX Level Decreased	0	0	1 (1.9)	1	1 (1.8)	1
Drug Ineffective	0	0	1 (1.9)	1	1 (1.8)	1
Eye Pruritus	0	0	1 (1.9)	1	1 (1.8)	1
Feeling Hot	0	0	1 (1.9)	1	1 (1.8)	1
Flushing	0	0	1 (1.9)	1	1 (1.8)	1
Hot Flush	0	0	1 (1.9)	1	1 (1.8)	1
Hypersensitivity	0	0	1 (1.9)	1	1 (1.8)	1
Infusion Site Reaction	0	0	1 (1.9)	1	1 (1.8)	1
Injection Site Pruritus	0	0	1 (1.9)	1	1 (1.8)	1
Lymphadenopathy	0	0	1 (1.9)	1	1 (1.8)	1
Lymphadenopathy Mediastinal	0	0	1 (1.9)	1	1 (1.8)	1
Myalgia	0	0	1 (1.9)	1	1 (1.8)	1
Nasopharyngitis	0	0	1 (1.9)	1	1 (1.8)	1
Night Sweats	0	0	1 (1.9)	1	1 (1.8)	1
Psoriasis	0	0	1 (1.9)	1	1 (1.8)	1
Pyrexia	0	0	1 (1.9)	1	1 (1.8)	1
Urticaria	0	0	1 (1.9)	1	1 (1.8)	1
Viral Infection	0	0	1 (1.9)	1	1 (1.8)	1

TEAE = treatment-emergent adverse event; [a] **Related or possibly related to study treatment**. Notes:

1. Treatment-emergent adverse events were adverse events with onset date on or after the date of etranacogene dezaparvovec administration.

2. Preferred terms are presented by descending incidence among subjects in both studies combined and by descending incidence in CT-AMT-061-02, then CT-AMT-061-01 for ties. Source: ISS safety update Table 3.4.1.2

One of the most common treatment-related adverse events was influenza like illness (ILI), which occurred in close temporal relationship after IP administration in several patients. The applicant was asked to comment whether the Investigators used a certain ILI definition (e.g., WHO, CDC) and to discuss a potentially increased risk for infection after administration of gene therapy. It was unclear if these reports were alternatively rather events of 'feeling sick' with different symptoms. The applicant clarified that no certain ILI definitions were used. It was further pointed out that for all events of ILI (by PT) the verbatim terms of 'flu like symptoms', 'flu like symptoms without fever', or 'feeling fluish' were used. All events were mild, most events occurred in close temporal relationship and resolved within 1 to 2 days of onset.

Part of transaminase increases and CK increases were assessed as related as well as part of arthralgia and other kinds of pain. Related increases in ALT, AST and CK were reported for 15.8%, 8.8% and 7.0% of subjects respectively. Some cases of arthralgia (3 subjects), as well as sporadic cases of pain (2 subjects), abdominal pain upper (1 subject), back pain (1 subject), myalgia (1 subject) were assessed as related. Even one viral infection was assessed as related as well as sporadic cases of diarrhoea, malaise, anaemia, abdominal discomfort etc. However, all related AEs have not been mentioned in the tabulated list of adverse events in SmPC section 4.8. The applicant was asked for clarification regarding the principle in defining the relationship of adverse events to the study treatment and the principle of choosing related adverse events to the list of ADRs in SmPC section 4.8.

The applicant clarified that after the adverse drug reaction (ADR) selection process was conducted (including screening of AEs, expert medical review, and thorough causality assessment), reasonable evidence of a causal relationship between several AEs and the IP could not be established for many TEAEs commonly reported by the investigators as related or possibly related to etranacogene dezaparvovec. Therefore, not all the TEAEs reported by investigators as related have been included in the table of ADRs in the SmPC.

The most common reasons for not including TEAEs in the ADR table are the following:

- A robust causal association with etranacogene dezaparvovec could not be established by the applicant
- The presence of strong confounding factors or the presence of more plausible alternative explanation (eg, underlying hemophilia B and / or its complications)
- No plausible explanation of the TEAE based on the known mechanism of action of etranacogene dezaparvovec and the pathogenesis of the AE.

The following TEAEs commonly reported as related by the investigator but not included in the tabulated list of AEs in SmPC Section 4.8 (eg, Arthralgia, Pain, Abdominal Discomfort, Back Pain, Myalgia, Unconfirmed Viral Infection, Diarrhoea, and Anaemia) met one or more of the reasons chosen by the applicant for not including such AEs in the ADR table of the SmPC.

It should be noted that "Abdominal Pain Upper" was proposed to be included as individual symptom under "Infusion-related Reaction" in the ADR table of the SmPC, and the ADR of "C-reactive Protein Increased" was subsequently added (as clarified in the response to Question 122).

In conclusion, it is considered, that the current list of ADRs included in the SmPC is adequate for the purpose of informing the prescriber of expected AEs that have a reasonable causal association with etranacogene dezaparvovec administration.

In the Integrated summary of clinical safety, there were no thrombotic events reported. In the HOPE-B study report, there were at least 1 myocardial infarction, one peripheral artery occlusive disease and one TIA (transient ischemic attack). According to the AE Listing, the myocardial infarction occurred during the lead-in period and the participant did not receive treatment with AMT-061. The applicant was asked to list all possible thromboembolic events in both studies and discuss their possible relation to study treatment.

The applicant explained that there were four TEAEs in 3 subjects identified as potentially relating to a thromboembolic event in Study CT-AMT-061-02. These were PTs of Angina Pectoris (2 events), Peripheral Arterial Occlusive Disease (1 event) and Transient Ischaemic Attack (1 event). The cases of Angina pectoris and Peripheral Arterial Occlusive Disease were assessed by the investigator as not related to IP and 1 serious TEAE of Transient Ischaemic Attack was assessed by the investigator and the Sponsor as unlikely related to IP.

The subject with nonserious TEAE of Peripheral Arterial Occlusive Disease (Day 547 postdose) was in the age group from 65 to 74 years of age and had a relevant medical history of aortic arteriosclerosis (from 2014 ongoing), and hypercholesterolaemia and hypertension.

The subject with nonserious TEAE of Angina Pectoris (Day 220 postdose) had also a serious TEAE of Transient Ischaemic Attack (Day 229 postdose). At the time of enrolment to Study CT-AMT-061-02, the subject was in the age group from 65 to 74 years of age and had a relevant medical history of Transient Ischaemic Attack from 2018, which resolved before enrolment in the study.

The other subject with a nonserious TEAE of Angina Pectoris (study day postdose not reported) was in the age group from 75 to 84 years of age and had a relevant medical history of hypertension for more than 25 years, atrial fibrillation and atrial enlargement. The subject later had a fatal serious TEAE of Cardiogenic Shock (Day 463 postdose, end date Day 464 postdose) following a nonserious TEAE of Urinary Tract Infection that progressed to Urosepsis (Day 463 postdose).

Additionally, one subject had an SAE of myocardial infarction during the lead-in period and the subject was not treated with etranacogene dezaparvovec as he met Exclusion Criterion #8 and was not eligible for inclusion in the study.

The subjects described above had relevant vascular and cardiac medical histories preceding the thromboembolic events. Also, these subjects belong to an aging population who are more likely to experience cardiac and vascular events.

With the data update, one additional treatment-related TEAE has been reported (PT: Lymphadenopathy Mediastinal) within the additional 6 months of safety follow-up. Upon request, the applicant clarified that the TEAE of Lymphadenopathy Mediastinal (moderate in severity, ongoing at the time of the 24-month data cut-off) was observed as an incidental finding on a CT scan performed for evaluation of the vascular system after an aneurysm of iliac artery was found. The Investigator confirmed that the subject did not have overt signs of pulmonary infection / lymphoma; the mediastinal nodes were > 15 mm in size and had a benign appearance. The Investigator considered that a causal relationship between etranacogene dezaparvovec and the TEAE of Lymphadenopathy Mediastinal was at least a reasonable possibility due to the lack of a specific diagnosis of an alternative root cause and the unknown long-term effects of a GTMP. The subject is still enrolled in the study and will continued to be followed as per the clinical study protocol.

Table 23. Incidence of Treatment-emergent Adverse Events Related to Study Treatment by Maximum Severity (ISS Safety Population)

		Study CT-AMT-061-01 (N = 3)	Study CT-AMT-061-02 (N = 54)	Total Etranacogene Dezaparvovec (N = 57)
Any TEAE Related to Study Treatment[a]	**Maximum Severity**	**n (%)**	**n (%)**	**n (%)**
	Mild	1 (33.3)	26 (48.1)	27 (47.4)
	Moderate	0	11 (20.4)	11 (19.3)
	Severe[b]	0	1 (1.9)	1 (1.8)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; TEAE = treatment-emergent adverse event.
[a] Related or possibly related to study treatment.
[b] One subject reported TEAEs related to study treatment that were assessed as severe: ALT Increased and AST Increased (each reported once).
TEAEs were adverse events with onset date on or after the date of etranacogene dezaparvovec administration.

Treatment-emergent Adverse Events (TEAEs) by Anti-AAV5 nAb Status at Baseline

The 33 subjects in the ISS Safety Population who were seronegative at baseline for anti-AAV5 nAb experienced 325 TEAEs; common TEAEs included Headache (12 [36.4%] subjects, 22 events), Arthralgia (11 [33.3%] subjects, 22 events), ALT Increased (8 [24.2%], 9 events), Fatigue (9 [27.3%] subjects, 11 events), Nasopharyngitis (8 [24.2%], 12 events), COVID-19 (7 [21.2%], 7 events), Toothache (6 [18.2%], 10 events), AST Increased (5 [15.2%], 6 events), Back Pain (5 [15.2%], 5 events), and Hypertension (5 [15.2%], 5 events). Of these 33 subjects, 22 (66.7%) had 57 treatment-related TEAEs. Most frequently reported treatment-related TEAEs by PT experienced by subjects within this subject group included ALT Increased (6 [18.2%], 7 events), Headache (6 [18.2%], 7 events), AST Increased (4 [12.1%], 5 events), Dizziness (3 [9.1%], 3 events), Fatigue (3 [9.1%], 3 events), and Influenza-like Illness (3 [9.1%], 4 events).

Twenty-four subjects who were seropositive for anti-AAV5 nAb at baseline experienced 288 TEAEs; common TEAEs included Arthralgia (10 [41.7%], 15 events), Nasopharyngitis (7 [29.2%], 8 events), Headache (6 [25.0%], 13 events), Back Pain (6 [25.0%], 9 events), Pain in Extremity (6 [25.0%], 7 events), Blood Creatine Phosphokinase Increased (5 [20.8%], 6 events), Fatigue (5 [20.8%], 6 events), Influenza-like Illness (4 [16.7%], 7 events), Diarrhoea (4 [16.7%], 4 events), Nausea (4 [16.7%], 4 events), and Oropharyngeal Pain (4 [16.7%], 4 events). Of the 24 subjects who were seropositive for anti-AAV-5 nAb at baseline, 17 (70.8%) experienced 38 treatment-related TEAEs. The common treatment-related TEAEs by PT experienced by subjects within the seropositive subgroup were comparable with those experienced in the seronegative subgroup, including Influenza-like Illness (4 [16.7%], 4 events), Headache (3 [12.5%], 3 events), ALT Increased (3 [12.5%], 3 events), C-reactive Protein Increased (3 [12.5%], 3 events), and Infusion Related Reaction (3 [12.5%], 3 events). No increased risk was identified for the 1 subject with a very high anti-AAV5 nAb titre of 3,212.

Treatment-emergent Elevations in Transaminases and Corticosteroid Use for Elevated Transaminases

In the ISS Population, 12 participants (21.1%) had 14 TEAEs of ALT Increased and 9 participants (15.8%) had 10 TEAEs of AST Increased. Most of these TEAEs were mild or moderate in severity, but 1 subject had elevations in AST and ALT that were reported as severe. Two subjects (one subject in Study CT-AMT-061-01 and one subject in Study CT-AMT-061-02) each had a TEAE with PT of transaminases increased; in 1 of the Subjects, the event occurred 218 days after infusion and was reported as 'elevated AST, ALT' and in the other Subject, the event occurred 740 days after infusion

and was reported as 'alcohol related transaminase increase'. No subject met the definition of drug-induced liver injury (DILI).

Nine subjects (15.8% of the ISS Population) used systemic corticosteroids for transaminase elevations in the Post-treatment Follow-up Period of Study CT-AMT-061-02. The mean corticosteroid treatment duration for those subjects was 79.8 days [range 51 to 130 days]. These nine subjects received steroids as treatment for the liver enzyme elevations of either > ULN (n = 8) or > 2 × baseline value (n = 1), including prednisone, prednisolone, and methylprednisolone. Five of the subjects receiving steroids had an isolated ALT increase and 4 subjects had both an elevation of ALT and AST. All transaminase elevations that were treated with steroids had an onset within 3 months post dose, with the earliest onset at Week 3. All subjects discontinued steroid use before Week 26.

All TEAEs regarding elevated transaminase were non-serious and resolved. One subject in study CT-AMT-061-01 had moderate TEAEs of ALT increased, AST increased, and blood creatine phosphokinase increased between Days 787 and 806 that resolved without treatment.

One subject had a mild AE of bilirubin increased that resolved within 9 days.

Table 24. Concomitant Systemic Corticosteroid Use (ISS Safety Population)

Parameter	Study CT-AMT-061-01 (N = 3)	Study CT-AMT-061-02 (N = 54)	Total Etranacogene Dezaparvovec (N = 57)
Any concomitant systemic corticosteroid[a], n (%)	2 (66.7)	20 (37.0)	22 (38.6)
Unrelated to etranacogene dezaparvovec [b], n (%)	2 (66.7)	11 (20.4)	13 (22.8)
Oral	1 (33.3)	2 (3.7)	3 (5.3)
Intravenous	0	7 (13.0)	7 (12.3)
Intra-articular	1 (33.3)	2 (3.7)	3 (5.3)
Intramuscular	0	2 (3.7)	2 (3.5)
Soft Tissue	1 (33.3)	0	1 (1.8)
Intrasynovial	0	1 (1.9)	1 (1.8)
Other	1 (33.3)	1 (1.9)	2 (3.5)
Associated with etranacogene dezaparvovec intravenous infusion[c], n (%)	0	3 (5.6)	3 (5.3)
Associated with transient transaminitis[d], n (%)	0	9 (16.7)	9 (15.8)
Duration of use associated with transient transaminitis [e] (days)			
N		9	9
Mean (SD)		79.8 (26.63)	79.8 (26.63)
95% CI for the mean		59.3, 100.2	59.3, 100.2
Median		74.0	74.0
IQR		57.0, 101.0	57.0, 101.0
Min, Max		51, 130	51, 130

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; IQR = interquartile range; Max = maximum; Min = minimum; SD = standard deviation.

[a] Corticosteroids, including glucocorticoids, that would potentially have systemic absorption and effect, and were manually flagged by the Sponsor.

[b] Not associated with either day of infusion or management of adverse event of transaminitis.

[c] Use in subjects on the day of administration in association with reported infusion-related reaction.

[d] Use in a tapered regimen initiated by an adverse event report of elevated ALT / AST.

[e] Duration for each medication is defined as [end date – start date + 1]; for each subject, total duration was calculated as [the sum over all medications – 1] for each day that was counted twice. The 95% confidence interval for mean duration was calculated using the t statistic.

Table 25. Listing of TEARs of ALT Increased and AST increased (Study Safety Populations)

Subject	Preferred Term	TEAE Severity	Relationship	Elevation > 2 × baseline	Treatment
Study CT-AMT-061-01					
[redacted]	ALT Increased	Mild	Not related	No	None
	ALT Increased	Moderate	Not related	Yes	None
	AST Increased	Moderate	Not related	Yes	None
Study CT-AMT-061-02					
[redacted]	ALT Increased	Moderate	Related	Yes	Prednisolone
[redacted]	AST Increased	Mild	Related	No	None
[redacted]	ALT Increased[a]	Moderate	Related	Yes	Prednisone
	AST Increased	Mild	Related	Yes	Prednisone
[redacted]	ALT Increased	Moderate	Related	Yes	Prednisone, Prednisolone
[redacted]	ALT Increased	Mild	Not related	No	Prednisone
[redacted]	ALT Increased	Mild	Not related	Yes	None
	AST Increased	Mild	Not related	Yes	None
[redacted]	ALT Increased[b]	Mild	Related	No	Prednisone
	AST Increased	Mild	Not Related	No	None
[redacted]	AST Increased	Mild	Related	No	None
	ALT Increased	Mild	Related	Yes	None
[redacted]	ALT Increased	Moderate	Related	Yes	Methylprednisolone
[redacted]	AST Increased	Mild	Related	Yes	None
	AST Increased	Severe	Related	Yes	Prednisone
	ALT Increased	Severe	Related	No	Prednisone
[redacted]	ALT Increased	Mild	Related	Yes	Prednisolone
	ALT Increased	Moderate	Related	Yes	Prednisolone
	AST Increased	Mild	Related	No	None
[redacted]	ALT Increased	Mild	Related	No	Prednisolone
[redacted]	AST Increased	Mild	Not related	Yes	None

ALT = alanine aminotransferase; AST = aspartate aminotransferase; TEAE = treatment-emergent adverse event.
[a] ALT on Study Day 24: 227 U/L (normal range: 6 to 41 U/L); Grade 3 per National Cancer Institute Common Terminology Criteria for Adverse Events. Subject also had a mild, treatment-related TEAE of blood bilirubin increased from Study Day 43 to 51 (bilirubin on Study Day 43: 21.9 µmol/L [normal range: 1.7 to 18.8 µmol/L]).
[b] ALT values were within normal range from Study Day 44 on.
[c] Subject had a moderate, not-related TEAE of blood creatine phosphokinase increased from Study Day 40 to 49 (creatine kinase on Study Day 40: 2457 U/L [normal range: 25 to 210 U/L]).
Related = possibly related or related. Not related = unlikely related or not related.

Table 26. Summary of Liver Enzyme Elevation Adverse Events

Subject	Pre-dose anti-AAV5 NAb Titer	TEAE Preferred Term (Duration)	TEAE Severity	Relationship[1]	Elevation >2 × baseline	Treatment (Duration)
[redacted]	<LOD	ALT increased (Study Day 22 to 36)	Moderate	Related	Yes	Prednisolone (Study Day 22 to 85)
[redacted]	<LOD	AST increased (Study Day 22 to 29)	Mild	Related	No	None
[redacted]	41.3	ALT increased (Study Day 24 to 150)[2]	Moderate	Related	Yes	Prednisone (Study Day 24 to 106)
		AST increased (Study Day 24 to 150)	Mild	Related	Yes	Prednisone (Study Day 24 to 106)
[redacted]	<LOD	ALT increased (Study Day 30 to 50)	Moderate	Related	Yes	Prednisolone (Study Day 36 to 38); Prednisone (Study Day 38 to 86)
[redacted]	<LOD	ALT increased (Study Day 36 to 149)	Mild	Not related	No	Prednisone (Study Day 49 to 149)
[redacted]	<LOD	ALT increased (Study Day 120 to 127)	Mild	Not related	Yes	None
		AST increased (Study Day 120 to 127)	Mild	Not related	Yes	None
[redacted]	57.8	ALT increased (Study Day 28 to Day 44)[3]	Mild	Related	No	Prednisone (Study Day 31 to 46 for immune response against vector, and Study Day 46 to 147 for elevated ALT)[2]
		AST increased (Study Day 213 to 247)	Mild	Not related	No	None
[redacted]	<LOD	AST increased (Study Day 74 to 85)	Mild	Related	No	None
		ALT increased (Study Day 74 to 106)	Mild	Related	Yes	None
[redacted]	98.5	ALT increased (Study Day 35 to 42)	Moderate	Related	Yes	Methylprednisolone (Study Day 43 to 98)
[redacted]	<LOD	AST increased (Study Day 40 to 42)	Mild	Related	Yes	None
		AST increased (Study Day 43 to 47)	Severe	Related	Yes	Prednisone (Study Day 43 to 99)
		ALT increased (Study Day 43 to 47)	Severe	Related	No	Prednisone (Study Day 43 to 99)
[redacted]	<LOD	ALT increased (Study Day 41 to 43)	Mild	Related	Yes	Prednisolone (Study Day 41 to 170)
		ALT increased (Study Day 78 to 133)	Moderate	Related	Yes	Prednisolone (Study Day 41 to 170)
		AST increased (Study Day 85 to 99)	Mild	Related	No	None
[redacted]	<LOD	ALT increased (Study Day 59 to 71)	Mild	Related	No	Prednisolone (Study Day 61 to 134)
[redacted]	13.7	AST increased (Study Day 728 to 746)	Mild	Not related	Yes	None

Abbreviations: AAV5 = adeno-associated viral vector serotype 5; ALT = alanine aminotransferase, AST = aspartate aminotransferase; LOD = limit of detection; NAb = neutralizing antibody; TEAE = treatment-emergent adverse event.

1. Related = Possibly related or related; assessment by the Investigator.
2. ALT on Study Day 24: 227 U/L (normal range: 6 to 41 U/L); Grade 3 per National Cancer Institute Common Terminology Criteria for Adverse Events. Subject also had a mild, treatment-related TEAE of blood bilirubin increased from Study Day 43 to 51 (bilirubin on Study Day 43: 21.9 µmol/L [normal range: 1.7 to 18.8 µmol/L]).
3. ALT values were within normal range from Study Day 44 on.
4. Subject had a moderate, not-related TEAE of blood creatine phosphokinase increased from Study Day 40 to 49 (creatine kinase on Study Day 40: 2457 U/L [normal range: 25 to 210 U/L]).

In relation to treatment of transaminase elevations, AEs Qualifying for Special Notification of Insomnia and Lymphocyte Count Decreased were reported. Insomnia occurred 2 days after initiation of prednisolone in 1 subject for a TEAE of ALT Increased. Prednisolone was tapered following resolution of the transaminase elevation and the TEAE of Insomnia resolved 6 days later. A TEAE of Lymphocyte Count Decreased occurred 3 days after initiation of prednisolone in another subject who had been treated for a TEAE of ALT Increased; the event of Lymphocyte Count Decreased resolved during prednisolone tapering.

The applicant was asked to clarify why the ALT increase in one subject was considered as not related to treatment with AMT-061. The duration of the event (day 36 to 149) and the fact that he received prednisone for the duration of 100 days (day 49 to 149) would suggest otherwise. The applicant responded that the reported causality assessment is the investigator's causality assessment, and that

no sponsor assessment of causality was made. The applicant agreed that time to onset of the AE, duration, and clinical course would suggest otherwise.

A subject experienced a treatment-related TEAE of ALT increased from day 28-44 (grade 1) and was treated with prednisone from day 46 to 147. Some months after tapering off the corticosteroid treatment, he reported an event of AST increased (day 213 to 247). a) The applicant was requested to clarify why this second event of transaminitis was considered as not related. b) With the submission of the new data package (24-month data), the applicant was asked comment whether there were cases of recurrence of transaminitis.

a) Regarding the relatively late (Day 213) occurring case of AST increased, the applicant described that a slightly increased value was detected by a local laboratory, while the central laboratory values were within the reference range. Elevated values for CRP and gamma glutamyltransferase were concomitantly detected. Additionally, the subject had isolated ALT/AST and gamma glutamyltransferase values greater than the upper limit of normal already during the lead-in study. Based on the provided information, it can be agreed that the relatively late event of slightly elevated AST might not have been caused by etranacogene dezaparvovec.

b) The applicant summarised available data for three additional participants with more than one event of ALT/AST increased. Based on the provided data, the currently available 2-year data does not raise a concern regarding reoccurrence or late onset of transaminitis after treatment with etranacogene dezaparvovec.

It was unclear why the events of ALT and AST increased in one subject were classified as severe, while the events in another subject were rated as mild (elevated AST) and moderate (elevated ALT). According to the applicant, the only grade 3 event was an ALT elevation 3 weeks post dose. The applicant was asked to comment. The applicant clarified that the discrepancies regarding the severity rating of elevated transaminases in both subjects were caused by initially discrepant guidance to investigators. The protocol was therefore amended in October 2020. The applicant further pointed out that the presentation and discussion of the clinical data consider the grading of the investigator.

2.6.8.3. Serious adverse event/deaths/other significant events

In the ISS Safety Population, 15 (26.3%) subjects experienced 18 treatment-emergent SAEs. Serious AEs with PT blood loss anaemia were reported for 2 (3.5%) subjects; no other SAEs were reported in more than 1 subject. No apparent pattern was notable in the SAEs reported.

No SAE was considered related to treatment. Of note, a SAE of osteonecrosis was reported for a participant with a medical history of osteonecrosis (diagnosis of the SAE: worsening of avascular necrosis – left hip).

There were two SAEs of blood loss anaemia, one event occurred in a participant who concomitantly experienced the SAE of Diverticulitis Intestinal Haemorrhagic (Preferred Term), while the second anaemia developed due to rectal bleeding from haemorrhoids.

A subject in the age group from 65 to 74 years experienced a transient ischaemic attack. The narratives for the participant revealed numerous risk factors and preconditions, including a TIA. The FIX activity levels had been stable during the course of the study in the range of 40-45%. Therefore, it can be agreed that increased FIX activity unlikely caused the TIA. In the narratives, it is speculated that a potentially pre-existing atrial fibrillation (AF) might have contributed to the TIA. However, the diagnosis for AF was made on Study Day 567. Overall, the Sponsor's assessment can be agreed, that the SAE of TIA appears unlikely related to AMT-061 due to several significant risk factors and preconditions, but a contributory role cannot be excluded.

Regarding a SAE of chest pain (grade 1, classified as serious due to hospitalisation), the applicant was asked to further substantiate why this event was considered as not related. The onset was one day after administration of AMT-061 and the subject was treated with nitroglycerin and amlodipine. The applicant described that the subject was treated with nitroglycerin and amlodipine, since he also experienced elevated blood pressure in addition to the chest pain. An electrocardiogram and chest x-ray did not identify signs of heart disease or pulmonary embolism. The pain in the chest wall was presumed to be thoracic muscle pain related to exercise. No further concerns are raised, although a potential relationship of this mild event with etranacogene dezaparvovec treatment cannot be excluded based on the close temporal relationship.

SAEs by anti-AAV5 Neutralizing Antibody Status at Baseline

Of the 33 subjects in the ISS Safety Population who were seronegative for anti-AAV5 nAb, 6 (18.2%) subjects experienced 6 treatment-emergent SAEs. Of the 24 subjects who were seropositive for anti-AAV5 nAb, 9 (37.5%) subjects experienced 12 treatment-emergent SAEs. The following SAEs were reported:

- seronegative at baseline: haemarthrosis, musculoskeletal chest pain, blood loss anaemia, COVID-19, epilepsy, nephrolithiasis;
- seropositive at baseline: diverticulitis intestinal haemorrhagic, blood loss anaemia, upper gastrointestinal haemorrhage, atrial fibrillation, cardiogenic shock, complication associated with device, cellulitis, jaw fracture, hepatocellular carcinoma, transient ischaemic attack, osteonecrosis, peripheral artery aneurysm.

All severe and the fatal SAEs were experienced by the anti-AAV5 nAb seropositive subjects except covid-19, which was experienced by a seronegative subject. The applicant was asked to discuss the fact that anti-AAV5 nAb seropositive subjects had twice as many SAEs as anti-AAV5 nAb seronegative subjects and all severe SAEs and the fatal SAE were experienced by the anti-AAV5 nAb seropositive subjects except Covid-19.

The applicant argued that five cases of severe SAEs in anti-AAV5 nAb seropositive subjects included a Jaw fracture, Upper gastrointestinal haemorrhage, Hepatocellular carcinoma, Cellulitis and Cardiogenic shock.

The jaw fracture followed an assault, the hepatocellular carcinoma has been evaluated thoroughly in other context, and the cardiogenic shock followed an urosepsis in an elderly patient. The Upper Gastrointestinal Haemorrhage was experienced by a subject who remained on prophylaxis therapy and had the highest titre of preexisting anti-AAV5 nAbs at baseline. There was also another subject who remained on prophylaxis with FIX replacement therapy, was anti-AAV5 nAb seropositive and experienced a bleeding-related serious (not severe) TEAE of Diverticulitis Intestinal Haemorrhage. It can be noted that both of these subjects who remained on prophylaxis had serious TEAEs attributed to the underlying disease, adding to the overall higher number of TEAEs in the subgroup of anti-AAV5 nAb seropositive subjects.

Based on the evaluation of serious TEAEs, it seems that positive anti-AAV5 nAb status at baseline does not raise safety concerns with etranacogene dezaparvovec treatment itself but may contribute to the need of remaining on prophylaxis with FIX replacement therapy.

Deaths

One death was reported in Study CT-AMT-061-02. One Subject aged between 75 and 84 years with a medical history of atrial enlargement, atrial fibrillation, and hypertension, experienced a fatal event of Cardiogenic Shock on Study Day 464, following a urinary tract infection. The Investigator considered

the event of Cardiogenic Shock as severe in intensity and unrelated to study medication. The Sponsor considered the event of Cardiogenic Shock as unrelated to study medication.

Of note, another death was reported for a participant of the supportive Study CT-AMT-060-01. According to the brief narratives, the patient was in the age group from 65 to 74 years at the time of enrolment and the case of death occurred after the (5-year) study period. The investigator assessed the relationship between AMT-060 and death as being unlikely related.

Treatment-emergent Hepatocellular Carcinoma

One SAE of hepatocellular carcinoma (HCC) was reported in Study CT-AMT-061-02 in a male subject aged between 65 to 74 years with multiple risk factors including a history of Hepatitis B, Hepatitis C, alcohol use, and fatty liver disease. The subject did not show evidence of significant fibrosis / cirrhosis or steatosis at screening or before treatment with etranacogene dezaparvovec. Chest computerised tomogram (CT) with angiography that included visualisation of the upper abdominal organs 2 weeks post treatment and liver ultrasound on Day 84 post treatment revealed no liver abnormalities.

On study Day 365, ultrasound per study protocol revealed a subcapsular lesion, prompting further assessment leading to the diagnosis of hepatocellular carcinoma.

On Study Day 443, surgical excision of the lesion, the surrounding tissue and of a second lesion discovered on intraoperative ultrasound was planned. However, the primary tumour was neither biopsied nor excised during the surgery due to the complex location, possible morbidity and the likelihood that excision would not impact prognosis of multifocal hepatocellular carcinoma. It is however noticed that a percutaneous biopsy was performed on Study Day 389.

The applicant was asked to clarify which lesions where biopsied and analysed. The applicant confirmed that specimens from the secondary lesion (in segment 2/3) and adjacent tissues (and not the primary tumour in segment 8) were used for integration analysis.

Results of the integration site (IS) analysis revealed 56 unique ISs in the HCC and 39 unique ISs in the HCC-adjacent sample respectively, which indicated that < 0.03% of the cells in the HCC and HCC-adjacent tissues had adeno-associated virus (AAV) integration. A dominant IS was not identified, as would be expected had the AAV vector integrated and led to clonal expansion of the tumour cells. Whole genome sequencing (WGS) identified five additional ISs and confirmed the lack of a dominant IS in the HCC sample. WGS also revealed genetic alterations on chromosomes 1, 8 and on the X-chromosome of the HCC sample, typical for HCCs. WGS and RNA sequencing indicated a pattern of gene expression in the HCC-adjacent sample more characteristic of a premalignant state than of healthy liver tissue. Finally, miRNA analysis identified genes known to be associated with the progression and development of HCC.

The Sponsor with support from an external expert group assessed that mutations in these genes are consistent with HCC-risk typical for patients with chronic hepatitis C, which had been present in this patient for years until HCV treatment. Based on these results it is concluded that while vector integration did occur to a minor degree, it is unlikely to have been causally related to the development of HCC in the study subject.

Regarding this case of hepatocellular carcinoma, the applicant was requested to address a number of questions (see the discussion on clinical safety section for more details).

Adverse Events Qualifying for Special Notification

Nineteen AEs Qualifying for Special Notification were reported in 12 (21.1%) subjects in the first 18 months of the Post-treatment Follow-up Period (ISS Safety Population). These 19 events coded to 17 separate PTs. The System Organ Classes with events that occurred in more than 1 subject were Injury,

Poisoning, and Procedural Complications (2 [3.5%]) and Nervous System Disorders (2 [3.5%]). The majority of these AEs were mild or moderate (18/19 events) in severity and considered treatment-related (14/19 events).

Seven subjects had TEAEs Qualifying for Special Notification related to IP administration; ie, Infusion Related Reaction (2 [3.5%]), Hypersensitivity (1 [1.8%]), Infusion Site reaction (1 [1.8%]), Dizziness (2 [3.5%]), Eye pruritus (1 [1.8%]), Flushing (1 [1.8%]), Headache (1 [1.8%]), Abdominal Pain Upper (1 [1.8%]), Urticaria (1 [1.8%]), Chest Discomfort (1 [1.8%]), and Pyrexia (1 [1.8%]). Three subjects with infusion reactions related to IP required a dose interruption. Five of the 7 subjects were positive for anti-AAV5 nAbs at baseline. The applicant was asked to show the titres of anti-AAV5 nAbs of those subjects having AEs related to IP administration and discuss whether anti-AAV5 nAbs increase the risk of AEs related to IP administration.

The applicant replied that four of these 5 subjects (positive for anti-AAV5 nAbs at baseline) had mild infusion-related reactions and 1 subject had a moderate infusion related-reaction. A higher nAb titre did not correlate with increased severity of AEs. Of the two subjects with AEs related to IP administration who were negative for anti-AAV5 nAbs at baseline, one had a mild AE and the other one had a moderate AE.

The need for treatment / action for the infusion-related reaction AEs was not predictable based on nAb positive status or titre; 1 subject who was negative for anti-AAV5 nAbs required concomitant medication and interruption of study drug, whereas the subject with the highest titre for anti-AAV nAbs did not require concomitant therapy or interruption of study drug and the subject with the second highest anti-AAV nAb titre of 1:678 did not experience an infusion-related reaction at all.

The applicant is of the opinion that there is currently insufficient evidence that the presence of preexisting anti-AAV5 nAbs would increase the risk of AEs related to IP administration as there is no correlation between the severity of infusion-related reaction AEs and anti-AAV5 nAb titres, and due to the small sample size. The risk of infusion-related reactions will be further characterised as part of post-marketing pharmacovigilance activities. This is acceptable.

One of the 7 subjects had a TEAE of Hypersensitivity. The event occurred during administration of etranacogene dezaparvovec and resulted in discontinuation of treatment and receipt of a partial dose (approximately 10%). The discontinuation of treatment in this subject occurred under the oversight and at the direction of a sub-investigator. A subsequent process review led to a protocol amendment which incorporated guidance for study sites on the management of infusion reactions. After implementation of the protocol amendment, no further treatment discontinuations occurred.

Three subjects had TEAEs Qualifying for Special Notification related to the development of any new / recurrent cancer. These included TEAEs of HCC (onset 365 days after etranacogene dezaparvovec treatment), Prostate cancer (onset: 350 days after etranacogene dezaparvovec treatment), and Basal Cell Carcinoma (onset: 550 days post dose), which were all assessed as not treatment-related. One subject had gastrointestinal lymphoma. The applicant was asked to justify why this lymphoma was not defined as an Adverse Event Qualifying for Special Notification and give more information of this subject.

The applicant clarified that this subject was in the age group between 65 to 74 years and had 2 findings from a routine colonoscopy on Day 203 postdose: nonserious TEAEs of Polyp and Gastrointestinal Neoplasm (nodule at ileocecal valve). On Day 420 postdose, the subject had another routine colonoscopy and a nonserious TEAE of Gastrointestinal Lymphoma (worsening of nodule at ileocecal valve) was reported. No information was provided about further diagnostic measures or treatment of the finding.

The study site was contacted for follow-up, and the investigator clarified that the subject had a diagnosis of Colon Adenoma (tubular adenoma of colon) since 2015 and not a new TEAE of Gastrointestinal Lymphoma. This information will be updated in the EDC system and the incorrect MedDRA coding will be revised accordingly.

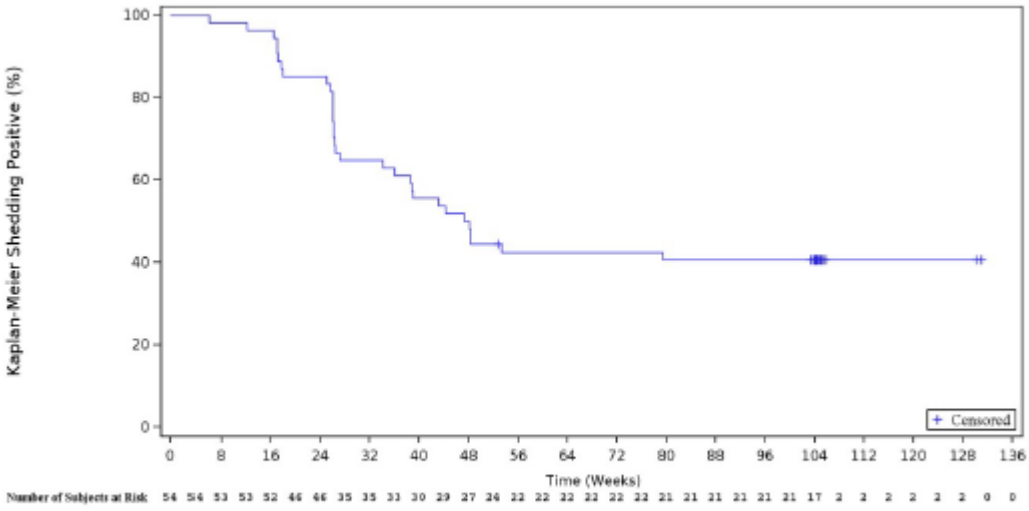
According to the applicant, the TEAE of Gastrointestinal Lymphoma was a coding error and no information indicated that this subject had a recurrent or new cancer. Consequently, this TEAE was appropriately not reported as an Adverse Event Qualifying for Special Notification.

2.6.8.4. Laboratory findings

Neither serum chemistry nor haematology parameters showed clinically meaningful variations from baseline after etranacogene dezaparvovec treatment, except elevated liver parameters in up to 18/57 (31.6%) patients. Hence, no safety concerns arise from serum chemistry or haematology observations. Clinically significant abnormalities regarding liver parameters and CRP and related TEAEs are discussed in detail above. Elevated liver parameters were included in section 4.8 of the SmPC and adverse events of elevated CRP levels were added upon request as well.

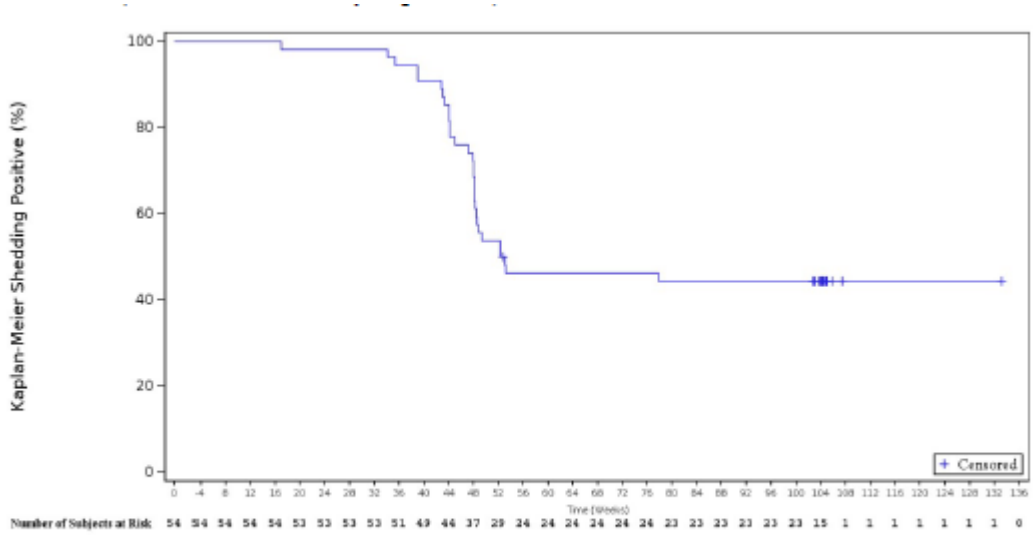
In the post-treatment period of Study CT-AMT-061-02 the absence of vector DNA shedding, defined as 3 consecutive samples with vector DNA levels < LOD, was confirmed in 37/54 (69%) and 30/54 (56%) subjects in semen and blood, respectively. The median time to absence of shedding was 52.3 weeks in blood and 45.8 weeks in semen at 24 months post-dose. Considering also shedding results obtained from the final 2 available consecutive samples, a total of 47/54 (87%) and 40/54 (74%) patients were identified to have reached absence of vector DNA from blood and semen, respectively, at 24 months post-dose. Upon request, the applicant has included a sufficiently detailed description of these observations in 5.2 of the SmPC.

Figure 5. Kaplan-Meier Curve for Time to First Vector Shedding Negative From Semen (Post-treatment Safety Population)



Shedding negative was defined as the first of 3 consecutive measures with a result below the limit of detection. Censoring time was truncated at the data cutoff date, the time of completion of the study, or time of early withdrawal from the study, whichever was earlier.

Figure 6. Kaplan-Meier Curve for Time to First Vector Shedding Negative From Blood (Post-treatment Safety Population)



Shedding negative was defined as the first of 3 consecutive measures with a result below the limit of detection. Censoring time was truncated at the data cutoff date, the time of completion of the study, or time of early withdrawal from the study, whichever was earlier.

In the absence of infectivity assays the duration of recommended contraception should be based on the available clinical study data. As the median time to vector shedding negative in semen was 45.8 weeks (95% CI 34.1, 52.1 weeks) and samples from 9 subjects were still positive for vector DNA at or after day 182 (updated Listing 3.8.2), the proposed recommended 6 months of double-barrier contraception were not considered sufficient and were extended to 12 months of barrier contraception. The applicant was asked to update section 4.6 of the SmPC to reflect that 12 months of barrier contraception are recommended.

Moreover, horizontal transmission is considered as an important potential risk due to declining levels of residual capsid and vector fragments shed through body fluids. The SmPC, Healthcare Professional Brochure and Patient Card should inform that patients treated with etranacogene dezaparvovec must not donate blood or organs, tissues and cells for transplantation. The requested information is currently included in 4.4 of the SmPC.

Levels of inflammatory markers IL-1β, IL-6, and MCP-1 were generally within the normal range after treatment with etranacogene dezaparvovec. Levels of IL-2 were transiently elevated in some patients but were below detection limit from Month 4. IFNγ levels were ≥LLOQ for the majority (44/54 [81.5%]) of subjects prior to dosing, and all patients had levels ≥LLOQ at least once following treatment. Overall, no new safety concerns arise since most inflammatory markers were unaffected or only transiently elevated.

AFP levels appeared unaffected by treatment with etranacogene dezaparvovec. Nevertheless, two subjects had elevated AFP: One subject had elevated baseline AFP and post-dose AFP and another subject had 1 elevated AFP value at Month 12. Upon request, the applicant provided the rationale why it is not necessary to control AFP levels in a yearly manner in all patients treated with etranacogene dezaparvovec. It was agreed that AFP levels alone would not provide adequate information, and that it is sufficient to recommend regular monitoring of AFP levels along with liver ultrasound screenings in patients with pre-existing risk factors for hepatocellular carcinoma only (SmPC 4.4). There were only minor changes in vital signs, the majority being mild to moderate in severity.

Shifts in abdominal ultrasound results from normal to abnormal occurred in 14/31 (45.2%). Upon request, reasons why some patients (26) had delayed baseline assessment (post-treatment) were not

sufficiently explained and it cannot be excluded that abnormalities observed in 11/26 subjects were absent before dosing and evolved/worsened after receiving Hemgenix. Upon request, the applicant presented more details for the 11 subjects with delayed or missed baseline ultrasound assessment, who had abnormal ultrasound results, in order to assess a possible relationship with Hemgenix. In 6/11 subjects a causal relationship between abnormalities reported and treatment-related TEAEs was ruled out due to the lack of biological plausibility. No treatment-related TEAEs were reported in 4/11 subjects. In the remaining subject, not clinically significant hepatic steatosis was present at the baseline ultrasound scan 37 days post-treatment, and treatment-related TEAE transaminitis was reported within the first 47 days post-dose, which is likely attributed to the treatment with etranacogene dezaparvovec. It cannot be excluded that the observed not clinically significant hepatic steatosis in this patient is also a result of treatment with etranacogene dezaparvovec. During the additional 6 months of follow up provided with the updated 2-year CSR, 5 subjects had shifts in ultrasound scan results from normal to abnormal, which were all considered not clinically relevant by the applicant. Monitoring of hepatic function to mitigate the risk of potential hepatotoxicity is appropriately reflected in Section 4.4 of the SmPC.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable

2.6.8.6. Safety in special populations

No clinically relevant safety differences by age, race, ethnicity, or BMI were noted in the 2 etranacogene dezaparvovec studies.

Of the 57 subjects treated with AMT-061, one subject was between 75 and 84 years of age and 6 subjects were between 65 and 74 years of age. The experience in elderly subjects is therefore limited and considered adequately reflected in section 4.2 of the SmPC.

Neoplasms were observed in 3/50 (6.0%) patients below 65 years of age (total 4 events) and in 3/7 (42.9%) subjects ≥65 years (total 7 events). Treatment-emergent SAEs were observed in 9/50 (18.0%) patients below 65 years of age and in 6/7 (85.7%) subjects ≥65 years. One SAE Hepatocellular Carcinoma was reported in one subject ≥65 years of age.

No unexpected differences in safety outcomes were observed between the age groups. More SAEs and neoplasms were observed in the elderly population, which were all considered not related to study drug, and may be expected in elderly patients. The SAE of Hepatocellular Carcinoma is described in the discussion section.

All subjects in Studies CT-AMT-060-01 and CT-AMT-060-02 were male. Therefore, no data exists on the effects of etranacogene dezaparvovec on pregnancy or lactation in a controlled clinical setting.

However, 2 pregnancies were reported in partners of male subjects enrolled in phase 1 Study CT-AMT-060-01, performed with AMT-060, the wild-type FIX predecessor of etranacogene dezaparvovec, resulting in viable, healthy offspring. Upon request, the applicant submitted the corresponding Development Safety Update Report (DSUR Version 8, reporting period 20-Aug-2020 to 19-Aug-2021, date of report 11-Oct-2021).

Animal studies with mice did not indicate any harmful effects with respect to reproductive toxicity.

Etranacogene dezaparvovec use has not been studied in breastfeeding women. It is not known whether etranacogene dezaparvovec is excreted in human milk. No clinical studies have been performed to evaluate the effects of etranacogene dezaparvovec on impairment of fertility. Effects on

male and female fertility have been evaluated in animal studies with mice. No adverse impact on the fertility was observed. "Use in female patients" was included as missing information in the safety specifications upon request (see the RMP section).

In absence of available data in females on the non-clinical and clinical levels, the section 4.6 of the SmPC was completed upon request, with some amendments suggested (see comment in SmPC). Although haemophilia is a rare condition in women, a strategy plan to eventually be able to treat women of childbearing potential with haemophilia B with etranacogene dezaparvovec in the future would be welcomed.

Table 27. Treatment-emergent Adverse Events by Age Group (Analysis Set: Safety Population)

MedDRA Terms	Age < 65 years (N = 50) N (%) E	Age 65 to 74 years (N = 6) N (%) E	Age 75 to 84 years (N = 1) N (%) E
Total AEs	50 (100) [514]	6 (100) [83]	1 (100) [16]
Serious AEs – Total	9 (18.0) [9]	5 (83.3) [7]	1 (100) [2]
- Fatal	0	0	1 (100)
- Hospitalization/prolong existing hospitalization	8 (16.0)	4 (66.7)	1 (100)
- Life-threatening	0	0	0
- Disability/incapacity	0	0	0
- Other (medically significant)	1 (2.0)	1 (16.7)	0
AE leading to drop-out	0	1 (16.7)	1 (100)
Infections and infestations	36 (72.0) [67]	5 (83.3) [12]	1 (100) [3]
Injury, poisoning and procedural complications	23 (46.0) [31]	3 (50.0) [6]	0
Nervous system disorders	23 (46.0) [57]	2 (33.3) [2]	0
Vascular disorders	9 (18.0) [10]	3 (50.0) [7]	1 (100) [1]
Sum of falls, dizziness, fractures	8 (16.0) [12]	1 (16.7) [1]	0
Psychiatric disorders	7 (14.0) [7]	0	0
Cardiac disorders	3 (6.0) [3]	2 (33.3) [3]	1 (100) [6]
Anticholinergic syndrome	NA	NA	NA
Quality of life decreased	NA	NA	NA

	Age 65 to 84 years (N = 7)
AEs appearing most frequently in older patients (> 20% of subjects [n ≥ 2/7 subjects] aged 65 to 75 years)	Abdominal pain upper
	Angina pectoris
	Atrial fibrillation
	Back pain
	Cystitis
	Fatigue
	Haemorrhoids
	Hypertension
	Influenza

AE = adverse event; E = number of events; MedDRA = Medical Dictionary of Regulatory Activities; N = number of subjects; NA = not applicable

2.6.8.7. Immunological events

Bioanalytical methods

At the beginning of clinical development the applicant has developed and validated an IgG and an IgM specific AAV-5 screening and titre assay. The missing confirmation assay was included into the ADA assessment strategy in a second development and assay validation campaign, prior to Phase III investigations. In brief, the screening assay consists of an ELISA, where AAV-5 coated to microtitre plates interacts with diluted serum. The specific IgG or IgM fraction is detected after a washing step by an anti-human IgG- or IgM-HRP conjugated antibody in presence of TMB and hydrogen peroxide. A cut point was set to reach a false positivity rate of 5% in the screening assay, based on 35 naïve individual human serum samples. Plate specific, floating cut-points were calculated by using a calibrator on each

plate. Positive samples only were further assessed for AAV-5 specific IgG or IgM titres using the same assay setup and eight linear dilution levels from 1:50 to 1:109350. Data points were fitted using a five-parameter logistic algorithm, and titres corresponded to the inverted dilution factor of the extrapolated intersection of the dilution curve with the assay cut-point. Both assays were validated for their precision, specificity and robustness, based on predefined acceptance criteria, which seem adequate, and which were met. Cut points were determined based on a 5% false positivity rate, which is recommended for screening assays. Assay precision, as well as selectivity and robustness were analysed and seem suitable for the intended application. Positive and negative controls as well as QC samples were properly determined. In 2019, a three tiered ADA assessment concept was adopted by implementing a confirmation assay, and all assays were re-evaluated. The presented approach in principle is acceptable.

The applicant has developed and validated a screening and confirmation ELISA for the assessment of human FIX specific immunoglobulins in human serum. In the screening assay human FIX coated to microtitre plates interacts with 1:50 diluted serum. FIX-specific Ig's are detected after a washing step by an anti-human IgG,A,M-HRP conjugated antibody in presence of TMB and hydrogen peroxide. A cut point was set to reach a false positivity rate of 5%, based on a sufficient number of individual human serum samples of naïve healthy individuals. The omission of a patient specific and FIX deficient matrix was justified by the applicant. Plate specific, floating cut-points were applied based on a human serum calibrator assessed on each plate, enabling to discriminate between anti-FIX positive and negative serum samples. To confirm positivity, samples which were screened reactive were assessed in the same setting, but pre-incubated with human FIX. For this confirmatory assay, a cut-point was defined based on 50 serum samples from naïve healthy donors, reflecting a false-positive rate of 1%. The set-up of both assays and the determination of assays specific cut-points seems appropriate. Both assays were initially validated for early clinical assessment, and re-validated in 2019 prior to Phase III studies. The chosen assay format lacks suitable human controls and validation was performed in a setting of limited relevance. Reported clinical anti-FIX levels were low, and confirmed by data from the anti-FIX neutralisation assay. Thus, the suitability of the assay for the intended purpose is supported. The validity of the assay was confirmed in human serum matrix.

The applicant has developed a luciferase- and a GFP-based HEK293//17 cellular neutralizing antibody assay, to assess the presence of AAV-5 specific neutralising antibodies. The use of cellular neutralisation assays is highly endorsed. Both have shown to detect the same entity, i.e. AAV5-neutralizing antibodies. Of the two, the luciferase-based assay appears to be the more sensitive, and was further applied in clinical trials. It was transferred and outsourced to Charles River, including banking of cell stocks. In brief, samples were analysed in seven 1:3 serial dilutions, and relative inhibitions were calculated for each dilution level. The calculated IC50 from the fitted relative inhibition curve is reported. The assay design including sample dilution, evaluation, positive and negative controls is considered state of the art, and seems acceptable. As outcome from an initial feasibility study, the assay cut-point was established based on a 1% false-positive rate, using 48 human serum samples from healthy donors. In a second validation study, assay performance regarding assay precision and reproducibility as well as robustness (including sample storage stability and effects of potentially interfering substances like haemoglobin up to 6.7 g/l haemoglobin and lipids) were assessed and met pre-defied acceptance criteria. The assay was tolerant to described substances, clinical samples were stable for 14 months, and the murine control antibody for 10 months. Eventually the assay was evaluated for performance at Precision for Medicine, regarding its selectivity, specificity, cross-reactivity, analytical cut-point, range, precision, sensitivity, linearity, carry-over, and sample stability. Assay precision was assessed for three individual positive control serum samples at three concentration levels and a positive neutralisation control over 6 individual assay runs, by in total three different analysts. Validation samples consisted of a murine monoclonal human AAV-5 capsid protein specific antibody spiked into immune-depleted human serum, which seems adequate. The assay seems

qualified for the intended semi-quantification of anti-AAV5-specific neutralising antibodies in human serum samples. All critical reagents and their provenience were described and cell banking reports were provided.

An IFNγ ELISpot Assay was developed and validated for the detection of AAV-5 Specific Cell Mediated Immunity. An initial validation of this method was performed with the purpose to ensure that the assay performs with adequate reproducibility and specificity, and that it is linear over a defined range. Validation of the IFNγ ELISpot assay for the detection of immune responses to AAV-5 whole virus was performed using peripheral blood mononuclear cells (PBMCs) from two different donors that have previously demonstrated a positive response towards AAV-5. The validation parameters included linearity, precision (reproducibility) and specificity. Many results did not fulfil the pre-determined criteria, especially for the PBMCs of one donor. It was concluded that the assay could still be useful and that the donors probably had a low number of precursor anti-AAV5 cells and that resulted in low number of SPF that would explain the high variability observed.

A second generation IFNγ ELISpot validation for AMT-061 with PBMC Lot 13830 was carried out. The response of Accucell™ cryopreserved PBMCs previously shown to respond to AAV-5 stimulation (Lot 13830) was tested for precision, sensitivity, repeatability, and reproducibility.

This study successfully validated the IFNγ ELISpot assay for monitoring the cellular immune response to uniQure's adeno-associated virus type 5 (AA V-5) capsid gene therapy candidate AMT-061. The stimulation of PBMC Lot 13830 with AMT-061 Lot Al8POOI yielded results that were repeatable within a plate, across plates, and across operators. The lowest number of cells that could be plated to precisely detect antigen-reactive cells following stimulation was 300,000 cells per well. Accucell™ cryopreserved PBMC Lot 13830 yielded a specific response and can be included as a control sample in clinical sample testing from trials measuring responses to AMT-061 Lot Al8POOI.

The method was shown to perform well when used to measure the cellular immune response against AMT-061.

Immunogenicity

At baseline, in the ISS Safety Population, nearly half [24/57 (42.1%)] of the subjects were positive for anti-AAV5 nAbs, with a median titre of 1:39.20. In Study CT-AMT-061-02, by Week 3 post dose, nAb levels were positive for all (53/53 [100%]) subjects assessed (median titre: 1:8748 [range: 1:8748, 1:8748]; upper limit of quantification: titre = 8748) and remained elevated through Month 24 post dose. Upon request, the applicant confirmed that the rapid development of nAbs did not affect transgene expression as evidenced by the increase in FIX activity in all subjects regardless of AAV5 nAbs post dose, and would not be relevant for the safety of patients treated with etranacogene dezaparvovec.

Likewise, all patients converted to anti-AAV5 IgG positive latest 3 weeks post treatment with increasing titres, which persisted over at least 24 months. Most patients had transiently increased anti-AAV5 IgM between 1-3 weeks, with decreasing titres up to 12 months post treatment, when 10/53 (18.9%) subjects remained positive. At month 24 post treatment, 11/52 (21.2%) subjects were positive for anti-AAV5 IgM. As expected, mean FIX activity levels post-treatment were higher in patients without pre-existing AAV nAbs compared to patients with pre-existing AAV nAbs.

In the ISS Safety Population, the majority (56/57 [98.2%]) of subjects tested negative for anti-FIX antibodies at baseline before etranacogene dezaparvovec dosing. One subject was positive for anti-FIX antibodies at baseline in study CT-AMT-061-01 in the screening assessment, but not in the confirmation assessment. Both assessments were negative by month 24. The subject's FIX activity levels were 37.9% at Week 12, 51.0% at Week 26, 50.2% at Week 52 and 41.5% at Month 36, thus no considerable reduction in FIX activity levels was observed at any time point. In study CT-AMT-061-

02 one subject tested positive for anti-FIX antibodies prior to dosing and post-treatment to month 6. The subject's FIX levels appeared at the lower end (8.4% at Month 6, 11.4% at both Month 12 and Month 18, and 10.1% at Month 24 post-treatment), but was temporally independent of the occurrence of anti-FIX antibodies.

Reassuringly, no FIX inhibitors were observed up to 24 months post-treatment. Subjects with FIX inhibitors have been excluded from the studies. The possible occurrence of FIX inhibitors should further be monitored for a total follow-up length of 10 years.

Availability of results on AAV capsid specific T cell responses is limited, since there were missing data due to issues related to insufficient number of cells and nonconformance in the analysis. Yet, the majority of subjects [39/54 (72.2%) in Study CT-AMT-061-02] had at least one time point with detectable T cell response. The highest number of detectable T cell responses occurred at week 6 [15/38 [39.5%] subjects in Study CT-AMT-061-02], which is slightly earlier than would typically be expected from gene therapy products (7-10 weeks). According to the applicant, the majority of T cell responses were transient and did not lead to a decrease in FIX activity. 6/39 (15.4%) subjects had concurrent TEAEs of ALT Increased and / or AST Increased, and of those 2 received corticosteroid treatment. Increased liver enzymes are expected following T cell responses to rAAVs that target the liver as expression system.

2.6.8.8. Safety related to drug-drug interactions and other interactions

No interaction studies have been performed, as no interactions are to be expected from the endogenous protein hFIXco-Padua. However, owing to its mode of action, potential liver toxicity following the administration of etranacogene dezaparvovec may occur. Upon request, the applicant added detailed information to 4.5 of the SmPC pertaining to monitoring of concomitant medications, as well as ALT and FIX activity after etranacogene dezaparvovec treatment. Furthermore, it is advised to avoid potentially hepatotoxic medications or other hepatotoxic agents and both, the risk of reduced efficacy and an increased safety risk, are highlighted. Prescribers are also made aware of medications potentially impacting corticosteroid treatment, as well as potential interactions of vaccines with immunomodulatory therapy.

2.6.8.9. Discontinuation due to adverse events

One subject prematurely discontinued from treatment due to a TEAE of Hypersensitivity (received partial dose). The subject continued in the study for follow-up.

2.6.8.10. Post marketing experience

Not applicable.

2.6.9. Discussion on clinical safety

The safety database of AMT-061 (etranacogene dezaparvovec) to support a market authorisation to treat adults with haemophilia B consists of data from two trials: 3 subjects received AMT-061 in Study CT-AMT-061-01 (an ongoing, 5-year, phase 2b, open-label, single-dose, single-arm, multicentre study); and 54 subjects received AMT-061 in Study CT-AMT-061-02 (an ongoing, pivotal, phase 3, open-label, single-dose, single-arm, multinational study, which included a ≥ 6-month Lead-in Period with standard of care continuous FIX prophylaxis).

The safety data from these 57 participants were combined in the Integrated Summary of Safety (Integrated Summary of Safety [ISS] Population). Of note, one subject received only 10% of his designated dose due to a hypersensitivity reaction during infusion. Supportive data with 5-year safety follow-up are available from 10 subjects who received the predecessor product AMT-060 (expressing WT human Factor IX) during study CT-AMT-060-01.

Overall, the number of recruited subjects and the safety database are very limited. However, the clinical development programme was discussed and agreed by SAWP/CHMP during several EMA Scientific Advice (EMA-SA) procedures (e.g., EMA/CHMP/SAWP/301451/2018).

The study population included in the clinical trials is considered acceptable for the safety assessment of AMT-061 in this rare disease. Due to the small sample size, subpopulations like elderly patients or patients with HIV are not sufficiently represented to allow any statements regarding a potentially different response and treatment of these subgroups.

In total, 57 subjects were followed-up for 1547.5 person-months post-dose. 54 participants have a duration of exposure >24 months. One subject died on study day 464.

Following an initial data submission, the applicant provided a safety data update upon request, including additional 6 months of data. Within the period until the new data cut-off, there have been no deaths, no related SAEs, 3 unrelated SAEs, no further reports of cancer, 2 AEs of Transaminase Increased (both considered not related to study treatment), no further use of steroids for elevated transaminases, and no FIX inhibitors detected. No increased rate of TEAEs was noted (99 new TEAEs were reported during the additional 6 months of follow up vs. 514 TEAEs reported during the first 18 months post-dose).

Adverse Events

Lead-in Period

Of the 54 subjects who finished the lead-in period (and received treatment with AMT-061), 37 (68.5%) reported 87 AEs, with nasopharyngitis (n=8, 14.8%) and arthralgia (n=4, 7.4%) as the most frequently reported AEs. Overall, it appears that at least some AEs shown in safety tables for the lead-in period could have been caused by background disease or other pre-existing conditions. Considering that the participants received their routine standard of care FIX prophylaxis, some of them most likely for many years, the comparability of the safety profile between the lead-in period and the period after administration of the gene therapy may be limited (potential underreporting of AEs during the lead-in study). In addition, the duration of the lead-in period was also considerably shorter (≥6 months) than the follow-up time until after administration of AMT-061, further limiting the comparability.

Treatment-emergent Adverse Events (TEAEs)

All participants in the ISS Safety Population (n=57) experienced at least 1 adverse event and a total number of 613 AEs were reported. The highest incidences of AEs were reported for the system organ classes (SOCs) of Infections and Infestations (73.7%), Musculoskeletal and Connective Tissue Disorders (68.4%), General Disorders and Administration Site Conditions (56.1%), Gastrointestinal Disorders (47.4%), Injury, poisoning and procedural complications (45.6%), Nervous System Disorders (43.9%), and Investigations (42.1%).

The highest subject incidences, irrespective of investigator causality assessment, were reported for the following TEAEs (by PT): arthralgia (36.8%), headache (31.6%), nasopharyngitis (26.3%), fatigue (24.6%), ALT Increased (21.1%), back pain (19.3%), COVID-19 (17.5%), pain in extremity (15.8%), AST increased (15.8%), and Blood Creatine Phosphokinase (CPK) increased (15.8%).

56 subjects (98.2%) reported 460 mild (grade 1) TEAEs, 39 subjects (68.4%) reported 135 moderate (grade 2) TEAEs, and 11 subjects (19.3%) reported 18 severe (grade 3) TEAEs. Serious Adverse Events are described in a section below.

The incidence of adverse events in the SOC of Neoplasms Benign, Malignant and Unspecified (including Cysts and Polyps) appears rather high for these small trials (6 subjects [10.5%] with 11 events, events described in the results section above). The following events were reported: adenoma benign, basal cell carcinoma, benign breast neoplasm, colon adenoma, gastrointestinal lymphoma, gastrointestinal neoplasm, hepatocellular carcinoma, meningioma, pancreatic neuroendocrine tumour, prostate cancer, and skin papilloma. According to the Investigator, all events were considered unrelated (or "unlikely" related) to treatment. The applicant was requested to provide (updated) narratives for all AEs in the SOC of neoplasms, also for possible future events. The applicant summarised background information for observed TEAEs. Overall, the provided information do not raise a new safety concern. According to the applicant, the TEAE of Gastrointestinal Lymphoma was a coding error. The study site was contacted for follow-up and the investigator clarified that the subject had a diagnosis of Colon Adenoma (verbatim term: tubular adenoma of colon) since 2015 and not a new TEAE of Gastrointestinal Lymphoma (verbatim term: worsening of nodule at ileocecal valve). The applicant describes that no information indicated that this subject had a recurrent or new cancer.

The TEAE of Pancreatic Neuroendocrine Tumour (verbatim: Pancreatic lesion, possible neuroendocrine tumour) was reported. Upon request, the applicant clarified that a nonspecific nodular focus of uptake at the pancreatic body / tail was observed on a PET scan, which was performed to further assess the subject's colon adenoma. Subsequently, a dedicated pancreas CT protocol was performed and 3 follow-up CT scans did not reveal abnormal findings. Thus, the presence of a Pancreatic Neuroendocrine Tumour was not confirmed.

Treatment-related TEAEs

Based on the assessment of the Investigator, 95 treatment-related AEs were reported for 39 participants (68.4%) in the ISS Safety Population. Common treatment-related TEAEs by SOC experienced by subjects were General Disorders and Administration Site Conditions (19 [33.3%]), Investigations (13 [22.8%]), Nervous System Disorders (10 [17.5%]), and Gastrointestinal Disorders (8 [14.0%]). The most frequently reported treatment-related AEs (by PT) were ALT increased (9 subjects [15.8%] with 10 events), headache (9 subjects [15.8%] with 10 events), influenza-like illness (7 subjects [12.3%] with 8 events), AST increased (5 subjects [8.8%] with 6 events), Blood Creatine Phosphokinase increased (4 subjects [7%] with 6 events), dizziness, fatigue, nausea (each by 4 subjects [7%] with 4 events).

The reported treatment-related TEAEs were mostly mild (27 subjects [47.4%]) or moderate (11 subjects [19.3%]). One subject (1.8%) reported two severe treatment-related events (ALT and AST increased).

Treatment-emergent Adverse Events (TEAEs) by Anti-AAV5 nAb Status at Baseline

The applicant presented a comparison of the safety profile between subjects who were seropositive for anti-AAV5 nAb at baseline vs. those who were seronegative. If one would simply divide the number of events by the number of subjects, this results in a slightly higher rate of AEs per subject in the seropositive subgroup (mean 12 AEs per subject vs. mean 9.9 AEs per subject). However, there is no difference regarding treatment-related AEs (mean 1.6 per subject [seropositive] vs. 1.7 AEs per subject [seronegative]). The incidences for the events of ALT increased and AST increased were higher in seronegative subjects.

Interestingly, all treatment-related AEs of C-reactive Protein increased (3 events in 3 subjects) were reported in subjects who were seropositive for anti-AAV5 at baseline. This may indicate an increased

risk for excessive inflammation in subjects seropositive for anti-AAV5. One event (grade 2) had a duration from Day 77 until Day 183 post treatment. The applicant included this event as adverse drug reaction in section 4.8 of the SmPC.

Overall, no major differences were noted between the groups (seropositive vs. seronegative for baseline anti-AAV5 nAbs). However, the sample size is too limited to allow firm conclusions.

Hepatotoxicity

In the ISS Population, 12 participants (21.1%) had 14 TEAEs of ALT Increased and 9 participants (15.8%) had 10 TEAEs of AST Increased. One subject with a TEAE of AST Increased had an isolated event whereas in the remaining 6 subjects, the AST Increased occurred at the time of an ALT Increased. Five subjects had an isolated TEAE of ALT Increased. Two subjects each had a TEAE with PT of transaminases increased; in one Subject, the event occurred 218 days after infusion and was reported as 'elevated AST, ALT' and in the other Subject, the event occurred 740 days after infusion and was reported as 'alcohol related transaminase increase'. Most of these TEAEs were mild or moderate in severity, but 1 subject had elevations in AST and ALT that were reported as severe. No subject met the definition of drug-induced liver injury (DILI).

Nine subjects (15.8% of the ISS Population) used systemic corticosteroids for transaminase elevations in the Post-treatment Follow-up Period of Study CT-AMT-061-02. The mean corticosteroid treatment duration for those subjects was 79.8 days [range 51 to 130 days]. These nine subjects received steroids as treatment for the liver enzyme elevations of either > ULN (n = 8) or > 2 × baseline value (n = 1), including prednisone, prednisolone, and methylprednisolone. All transaminase elevations that were treated with steroids had an onset within 3 months post dose, with the earliest onset at Week 3. All subjects discontinued steroid use before Week 26.

All TEAEs regarding elevated transaminase were non-serious and resolved. One subject in study CT-AMT-061-01 had moderate TEAEs of ALT increased, AST increased, and blood creatine phosphokinase increased between Days 787 and 806 that resolved without treatment.

Several questions were raised, including the assessment of severity and/or relatedness of certain cases of enzyme elevations, and the potential for recurrence of transaminitis after 24-month follow-up. The responses are summarised in the respective AE section further above.

Transiently elevated liver parameters are expected due to the mechanism of action of etranacogene dezaparvovec. Importantly, based on the provided information, the currently available 2-year data do not raise a concern regarding reoccurrence or late onset of transaminitis after treatment with etranacogene dezaparvovec. Hence, the clinical safety profile regarding potential hepatotoxicity could be considered acceptable.

Serious Adverse Events and deaths

In the ISS Safety Population, 15 (26.3%) subjects experienced 18 treatment-emergent SAEs. Of the 18 SAEs reported for the ISS Safety Population, 2 were mild (grade 1) in severity, 8 were moderate (grade 2), and 8 were considered as severe (grade 3). No SAE was considered related to treatment. According to information provided in the CSR, the Investigator initially considered the events of transient ischaemic attack (TIA) and HCC as "possibly related" to treatment. Upon further investigations, both events were reassessed as "unlikely related".

One death was reported in Study CT-AMT-061-02. This Subject was a White male aged between 75 to 84 years with a medical history of atrial enlargement, atrial fibrillation, and hypertension, that experienced a fatal event of Cardiogenic Shock on Study Day 464, following a urinary tract infection. The Investigator considered the event of Cardiogenic Shock as severe in intensity and unrelated to study medication. Based on the provided information and considering that the patient experienced

atrial fibrillation prior to enrolment (since 2019), no objection regarding the Investigator's assessment is made. However, it cannot be excluded that treatment with AMT-061 could have worsened the state of the patient.

Treatment-emergent Hepatocellular Carcinoma

One SAE of hepatocellular carcinoma (HCC) was reported in Study CT-AMT-061-02 in a male subject agreed between 65 to 74 years with multiple risk factors including a history of Hepatitis B, Hepatitis C, alcohol use, and fatty liver disease. The subject did not show evidence of significant fibrosis / cirrhosis or steatosis at screening or before treatment with etranacogene dezaparvovec. Chest computerised tomogram (CT) with angiography that included visualisation of the upper abdominal organs 2 weeks post treatment and liver ultrasound on Day 84 post treatment revealed no liver abnormalities. On study Day 365, ultrasound per study protocol revealed a subcapsular lesion. Upon fine needle aspiration, the lesion was subsequently confirmed as HCC.

Results of an integration site (IS) analysis were briefly described in the patient narratives. The applicant describes that numerous integration sites were detected (56 unique ISs in the HCC and 39 unique ISs in the HCC-adjacent sample respectively), but no dominant IS was identified, which would speak against the possibility of clonal expansion of tumour cells. For both HCC and HCC-adjacent samples, a high number of vector-vector fusion sequences was detected, demonstrating that approximately 1 out of 10,000 vector genomes were integrated in the host genome. Further, it is stated that miRNA analysis identified genes known to be associated with the progression and development of HCC, consistent with HCC-risk typical for patients with chronic hepatitis C.

The applicant was requested to provide further information on the case of HCC. Due to a largely successful trans-arterial chemoembolisation procedure, no residual primary tumour samples from segment 8 were available following explantation of the subject's liver. Therefore, several randomly chosen samples were procured for further analysis. These include vector copy number and FIX-Padua mRNA analyses. According to the applicant, no results were available at the time of the submission of the responses to the LoOI. The outcome of these analyses will be submitted once available. Additional integration site analyses are not planned as no identifiable tumour was present in the provided liver samples.

It was confirmed that specimens from the secondary lesion (in segment 2/3) and adjacent tissues (and not the primary tumour in segment 8) were used for integration analysis. The primary lesion was not excised due to the complex location of the lesion and procedure-related risk.

In addition, a comprehensive HCC analysis report was provided. The main messages from this report were already correctly presented in the patient narratives submitted with the initial data submission. These narratives already mentioned that the HCC sample as well as the HCC-adjacent sample revealed mutations in a variety of genes that have been previously associated with HCC, suggesting a premalignant state rather than healthy liver tissue. However, further details regarding the observed mutations were missing in the initial data package. The provided analysis report describes genomic alterations in a plethora of genes which have been associated with HCC.

Overall, the additional detailed information is considered supportive regarding the assumption that the patient's liver tissue could already have been in a premalignant state. However, it is unknown whether the gene therapy could to some extent have contributed to development of HCC.

Expert reports were also submitted. All three experts describe that the patient was at increased risk of developing hepatocellular carcinoma due to several viral and non-viral risk factors. Especially the genomic alterations commonly seen in HCC and literature suggesting an increased risk after HCV eradication were pointed out.

Additional other concerns were raised with regards to the relevance of published literature (e.g., LaBella et al 2020, Dalwadi et al 2020) for AMT-061. The applicant was asked to explain the strategy on how to gain further knowledge on the extent of vector integration after administration of AMT-061. It is acknowledged that performing liver biopsies only for the purpose of trying to obtain data on integration and tumorigenicity would not be justified. It is expected that in case of (hepatic) tumours, full genomic sequencing of possible tumours would be performed to substantiate the potential risk of integrative events in (hepatocyte) clones. In addition, the applicant was asked to commit to make available the option for genomic sequencing to be conducted on (hepatic) tumours if they emerge from any patients in the post-authorisation studies. According to the responses, the applicant intends to investigate potential cases of HCC in a similar manner as the HCC case during the AMT-061-02 trial (provided informed consent by the patients). The observational long-term follow-up study will include respective wording. Further, the applicant commits to make available the option for genomic sequencing to be conducted on potentially relevant tumours if they emerge from any patients in the post-authorisation studies. Adequate information has been included in section 4.4 of the SmPC.

The uncertainty regarding carcinogenicity cannot be resolved, since the follow-up duration during the trials is too short and the sample size too small. The currently available information suggests that there might be a lifelong risk of insertional mutagenesis and subsequently carcinogenesis. Therefore, it is of utmost importance that every potential recipient of the treatment is well-informed about these risks prior to administration. As included in the RMP, the applicant is expected to prepare a concise and well-understandable patient information document that informs about this irreversible risk prior to administration of Hemgenix. Additional risk minimisation measures (guide for healthcare professionals, patient guide and patient card) are requested in the RMP and Annex II–D.

Adverse Events Qualifying for Special Notification

Nineteen AEs Qualifying for Special Notification were reported in 12 (21.1%) subjects. These 19 events coded to 17 separate PTs and the majority of these AEs were mild or moderate (18/19 events) in severity and considered treatment-related (14/19 events).

Seven subjects experienced TEAEs Qualifying for Special Notification related to IP administration; ie, Infusion Related Reaction (2 [3.5%]), Hypersensitivity (1 [1.8%]), Infusion Site reaction (1 [1.8%]), Dizziness (2 [3.5%]), Eye pruritus (1 [1.8%]), Flushing (1 [1.8%]), Headache (1 [1.8%]), Abdominal Pain Upper (1 [1.8%]), Urticaria (1 [1.8%]), Chest Discomfort (1 [1.8%]), and Pyrexia (1 [1.8%]). Three subjects with infusion reactions related to IP required a dose interruption. Five of the 7 subjects were positive for anti-AAV5 nAbs at baseline. One of the 7 subjects had a TEAE of Hypersensitivity. The event occurred during administration of etranacogene dezaparvovec and resulted in discontinuation of treatment and receipt of a partial dose (approximately 10%). After implementation of a protocol amendment, which incorporated guidance for study sites on the management of infusion reactions, no further treatment discontinuations occurred.

The SmPC includes information regarding infusion-related dose interruptions (section 4.8), including potential treatment options based on clinical judgement (section 4.4), which is supported.

An event "drug ineffective" was reported by a participant who had by far the highest anti-AAV5 nAb titre at baseline.

Three subjects had TEAEs Qualifying for Special Notification related to the development of any new / recurrent cancer. These included TEAEs of HCC (onset 365 days after etranacogene dezaparvovec treatment), Prostate cancer (onset: 350 days after etranacogene dezaparvovec treatment), and Basal Cell Carcinoma (onset: 550 days post dose), which were all assessed as not treatment-related (see sections above).

Risk for thrombosis/thromboembolic events

There were four TEAEs in 3 subjects identified as potentially relating to a thromboembolic event in Study CT-AMT-061-02. These were PTs of Angina Pectoris (2 events), Peripheral Arterial Occlusive Disease (1 event) and Transient Ischaemic Attack (1 event). The cases of Angina pectoris and Peripheral Arterial Occlusive Disease were assessed by the investigator as not related to IP and 1 serious TEAE of Transient Ischaemic Attack was assessed by the investigator and the Sponsor as unlikely related to IP. The subjects described above had relevant vascular and cardiac medical histories preceding the thromboembolic events. Also, these subjects belong to an aging population above 65 years who are more likely to experience cardiac and vascular events (see the results section for more details).

Thromboembolic events are considered as an important potential risk in the RMP.

Laboratory findings

Currently, no safety concerns arise from serum chemistry or haematology observations. Elevated liver parameters, which were observed in up to 18 (31.6%) patients, are discussed in the context of related TEAEs and potential hepatotoxicity above.

Most inflammatory markers were unaffected (IL-1β, IL-6, and MCP-1) or only transiently elevated (IL-2, IFNγ). Two subjects had elevated AFP. Regular monitoring of AFP levels along with liver ultrasound screenings in patients with pre-existing risk factors for hepatocellular carcinoma is recommended (SmPC 4.4). Vital signs did not raise any safety concerns. Occurring shifts in ultrasound results from normal to abnormal [14/31 (45.2%)] do not raise specific concerns at the moment.

Vector DNA shedding

In the post-treatment period of Study CT-AMT-061-02 the absence of vector DNA shedding, defined as 3 consecutive samples with vector DNA levels < LOD, was confirmed in 37/54 (69%) and 30/54 (56%) subjects in semen and blood, respectively. The median time to absence of shedding was 52.3 weeks in blood and 45.8 weeks in semen at 24 months post-dose. Considering also shedding results obtained from the final 2 available consecutive samples, a total of 47/54 (87%) and 40/54 (74%) patients were identified to have reached absence of vector DNA from blood and semen, respectively, at 24 months post-dose. Upon request, the applicant has included a sufficiently detailed description of these observations in 5.2 of the SmPC. In the absence of infectivity assays the duration of recommended contraception should be based on the available clinical study data. As the median time to vector shedding negative in semen was 45.8 weeks (95% CI 34.1, 52.1 weeks) and samples from 9 subjects were still positive for vector DNA at or after day 182 (updated Listing 3.8.2), the proposed recommended 6 months of double-barrier contraception were not considered sufficient and were extended to 12 months of barrier contraception. This information was included in section 4.6 of the SmPC upon request. In addition, the SmPC appropriately informs that patients treated with etranacogene dezaparvovec must not donate blood or organs, tissues and cells for transplantation.

Safety in Special Populations

The experience in elderly subjects is limited and considered adequately reflected in section 4.2 of the SmPC. From the available data, no unexpected differences in safety outcomes were observed between the age groups. More SAEs and neoplasms were observed in the elderly population, which were all considered not related to study drug.

Two pregnancies were reported in partners of AMT-060 (the wild-type FIX predecessor of etranacogene dezaparvovec) treated subjects and no clinical sequelae in the mothers or newborns were noted. The applicant submitted the Development Safety Update Report (DSUR) upon request.

There is no clinical data regarding administration of etranacogene dezaparvovec to female subjects. Consequently, there is also a complete lack of clinical knowledge regarding fertility in humans or the question whether etranacogene dezaparvovec is excreted in human milk. "Use in female patients" was included as missing information in the safety specifications upon request (see the RMP section).

Although haemophilia is a rare condition in women, a strategy plan to eventually be able to treat women of childbearing potential with haemophilia B with etranacogene dezaparvovec in the future would be welcomed.

Immunological events

All patients converted to anti-AAV5 IgG positive and AAV nAb positive at the latest 3 weeks post treatment, and most patients had transiently increased anti-AAV5 IgM between 1-3 weeks. As expected, mean FIX activity levels post-treatment were higher in patients without pre-existing AAV nAbs compared to patients with pre-existing AAV nAbs. Upon request, the applicant confirmed that the rapid development of nAbs did not affect transgene expression regardless of AAV5 nAbs post dose, and would not be relevant for the safety of patients treated with etranacogene dezaparvovec.

One subject was positive for anti-FIX antibodies at baseline in study CT-AMT-061-01 in the screening assessment, but not in the confirmation assessment. Both assessments were negative by month 24. In study CT-AMT-061-02 one subject tested positive for anti-FIX antibodies prior to dosing and post-treatment to month 6. Both subjects' FIX levels were temporally independent of the occurrence of anti-FIX antibodies. No FIX inhibitors were observed up to 24 months post-treatment. The possible occurrence of FIX inhibitors should further be monitored in case increased plasma Factor IX activity levels are not achieved, decrease, or bleeding is not controlled or returns.

Availability of results on AAV capsid specific T cell responses is limited due to missing data related to insufficient number of cells and nonconformance in the analysis. From the available data, the majority of subjects had at least one time point with detectable T cell response, but most were transient and did not lead to a decrease in FIX activity. 6/39 (15.4%) subjects had concurrent TEAEs of ALT Increased and / or AST Increased, and of those 2 received corticosteroid treatment.

Drug-drug interactions

No specific interaction studies for etranacogene dezaparvovec have been performed. However, owing to its mode of action, potential liver toxicity may occur. Upon request, the applicant added detailed information to 4.5 of the SmPC pertaining to monitoring of concomitant medications, as well as ALT and FIX activity after etranacogene dezaparvovec treatment. Furthermore, it is advised to avoid potentially hepatotoxic medications or other hepatotoxic agents and both, the risk of reduced efficacy and an increased safety risk, are highlighted. Prescribers are also made aware of medications potentially impacting corticosteroid treatment, as well as potential interactions of vaccines with immunomodulatory therapy.

Supportive Safety Data from Study CT-AMT-060-01

The open-label, uncontrolled study CT-AMT-060-01 was conducted with two doses of AAV5-hFIXco (AMT-060), the predecessor of etranacogene dezaparvovec, and is therefore considered supportive only. The primary objective of study CT-AMT-060-01 was to assess the 5-year safety profile of AMT-060. Most TEAE were mild or moderate in severity, one SAE of myelopathy was categorised as severe, not treatment-related, and resolved by study completion. Three SAEs (hepatic enzyme increased, pyrexia, ALT increased) were considered treatment-related. Three TEAE qualifying for special notification involving increased liver parameters were reported and treated with corticosteroids. The safety data revealed no additional concerns, as increased liver parameters are expected due to the

mode of action of the IMP. All subjects stopped shedding vector DNA from blood and semen in study CT-AMT-060-01.

One death occurred outside the study period. The patient in the age group from 65 to 74 years was found lifeless on the living room floor and was considered to have died from a natural cause and no autopsy was requested or planned. The investigator assessed the relationship between AMT-060 and death as being unlikely related.

Overall, the safety and efficacy profile of AMT-060 would support a favourable benefit/risk balance of etranacogene dezaparvovec. The CSR for ongoing long-term extension study CT-AMT-060-04 is expected in September 2026.

In summary, based on the (limited) short to medium-term safety data, gene therapy with AMT-061 was relatively well tolerated by the majority of study participants. The most significant short-term safety concern are potential hypersensitivity reactions. Of note, one participant only received 10% of his designated dose due to hypersensitivity. Some other patients also reported infusion related reactions (e.g., urticaria, eye pruritus, flushing, dizziness, pyrexia), but received the full dose. For 3 patients the infusion was temporarily paused and resumed at a reduced infusion rate after treatment with antihistamines and/or corticosteroids. Considering that only one administration of AMT-061 is necessary, the risk of hypersensitivity appears manageable.

Currently, the most relevant medium-term safety concern seems to be the risk of experiencing elevated liver enzymes (ALT, AST), which may necessitate treatment with corticosteroids. Intake of corticosteroids over an extended period of time poses its own risk of developing adverse events. Of note, a nonclinical study suggests that liver injury increases the risk of HCC in mice who received AAV gene therapy (Dalwadi et al. 2020).

The potential risk of malignancy as a result of vector integration will remain an important uncertainty that can only be addressed by long-term (over decades or life-long) safety observation of a much larger number of patients who received AMT-061. Due to the very limited sample size, uncommon or rare events were most likely not captured by the presented clinical studies. This needs to be addressed by post marketing surveillance.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.10. Conclusions on the clinical safety

Therapy with AMT-061 was relatively well tolerated by the majority of patients. The most relevant short to medium-term safety concerns are hypersensitivity reactions during administration and elevated transaminases that may require treatment with glucocorticoids in some patients.

Biopsies from a patient who developed a hepatocellular carcinoma revealed vector integration in the tested samples, thereby confirming nonclinical findings. However, the patient had several non-viral and viral risk factors, including genomic alterations in several genes which have been associated with HCC, which were also found in HCC-adjacent tissues.

Upon request, the applicant provided a safety update with 6 months additional follow-up. The new safety data did not reveal a new safety concern.

Overall, the safety profile is considered acceptable. Diligent post marketing surveillance is of utmost importance to detect potential rare adverse events and to investigate the potential risk of malignancy (due to vector integration) on the longer term. Patients must be well-informed about this to receiving etranacogene dezaparvovec. In this regard, a warning has been added to the SmPC and package

leaflet to inform on the potential risk of malignancy as a result of vector integration in liver cells and in other body cells.

These aspects are covered in the SmPC and Package leaflet and in the educational materials in the RMP, as reflected in Annex II.

The CHMP endorses the CAT conclusion on clinical safety as described above.

2.7. Risk Management Plan

2.7.1. Safety concerns

Summary of safety concerns	
Important identified risks	• Hepatotoxicity • Infusion reactions (including hypersensitivity)
Important potential risks	• Risk of malignancy in relation to vector integration in the DNA of body cells • Bleeding as a result of lack of efficacy due to immune-mediated neutralisation of the AAV-5 vector capsid • Thromboembolic events • Germline transmission • Transmission to third parties (horizontal transmission) • Development of FIX inhibitors
Missing information	• Use in patients with severe hepatic impairment • Long-term effect • Use in female patients

2.7.2. Pharmacovigilance plan

Study Status	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
Not applicable.				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
Not applicable.				
Category 3 - Required additional pharmacovigilance activities				
CSL222_3003 An Extension Study Assessing the Long-term Safety and Efficacy of Etranacogene Dezaparvovec Previously Administered to Adult Male	Primary Objective To assess the long-term safety in adult male patients with haemophilia B who were treated with etranacogene	• Hepatotoxicity • Risk of malignancy in relation to vector integration in the DNA of body cells	Annual updates in the DSUR	DLP: 19 August

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Patients with Hemophilia B during the CSL222_2001 (CT AMT-061-01) and CSL222_3001 (CT AMT-061-02) Studies. Planned	dezaparvovec in Study CSL222_2001 or CSL222_3001. Secondary Objective To investigate the long-term efficacy profile in adult male patients with haemophilia B who were treated with etranacogene dezaparvovec in Study CSL222_2001 or CSL222_3001.	• Thromboembolic events • Development of FIX inhibitors • Long-term effect	Interim reports	3-yearly
			Final report	31 March 2036
CSL222_5001 Survey to evaluate the effectiveness of additional risk minimisation measures (aRMMs) for Hemgenix among prescribers in the EU. Planned	Objectives: 1. Assess (Healthcare Professionals') HCP's awareness of the aRMM tools by estimating the proportion of targeted HCPs who acknowledge receiving the tools. 2. Assess HCP's utilisation of the aRMM tools by estimating the proportion of targeted HCPs who acknowledge reading and utilizing the tools. 3. Assess HCP's knowledge and behaviour pertaining to the key risk messages detailed in the aRMM by estimating the proportion of targeted HCPs with correct responses to knowledge and behaviour questions pertaining to the key risk messages.	• Hepatotoxicity • Risk of malignancy in relation to vector integration in the DNA of body cells • Thromboembolic events • Germline transmission • Transmission to third parties (horizontal transmission) • Development of FIX inhibitors • Long-term effect	Start of data collection	12 months after commercial launch of Hemgenix. (Actual date to be determined)
			Annual updates	No interim analyses or progress reports are planned.
			Final report	6 months after end of the survey (Actual date to be determined)

In addition, the following studies imposed primarily for effectiveness reasons will also provide safety results:

Study Status	Summary of objectives	Efficacy uncertainties addressed	Milestones	Due Date
Efficacy studies which are conditions of the marketing authorisation				
CSL222_4001 An observational post-authorisation Long-term Follow-up Study to Characterize the Safety and Effectiveness of HEMGENIX (Etranacogene Dezaparvovec) in Patients with Hemophilia B	Primary Objective To investigate the long-term effectiveness profile in adults with haemophilia B who are treated with HEMGENIX or are on continuous FIX prophylaxis by following	Long term effect Safety concerns also addressed: •Hepatotoxicity	Protocol submission	31 March 2023
			Start of data collection	30 September 2023

Study Status	Summary of objectives	Efficacy uncertainties addressed	Milestones	Due Date
Planned	them for a period of 15 years. <u>Secondary Objective</u> To characterise the long-term safety in adults with haemophilia B who are treated with HEMGENIX or are on continuous FIX prophylaxis by following them for a period of 15 years.	•Infusion reactions (including hypersensitivity) •Risk of malignancy in relation to vector integration in the DNA of body cells •Bleeding as a result of lack of efficacy due to immune mediated neutralisation of the AAV-5 vector capsid •Thromboembolic events •Germline transmission •Transmission to third parties (horizontal transmission) •Development of FIX inhibitors •Use in patients with severe hepatic impairment •Use in female patients	Study progress reports	Annually
			Interim reports	3-yearly at 3, 6, 9, 12, 15 and 18 years
			End of data collection	Last patient 15 years post dose data collected (2043)
			Final study report submission	31 December 2044
Efficacy studies which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
CSL222_2001 / CT-AMT-061-01 A phase 2b, open-label, single-dose, single-arm, multi-center trial to confirm the Factor IX activity level of the serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimized human factor IX gene (AAV5 hFIX Padua) administered to adult subjects with severe or moderately severe Hemophilia B. Ongoing	<u>Primary objective</u> To confirm that a single dose of 2 x 10^{13} genome copies (gc)/kg AMT-061 will result in factor IX (FIX) activity levels of ≥5% at six weeks after dosing. <u>Secondary objective</u> To assess further efficacy and safety of 2 x 10^{13} gc/kg AMT-061.	Long term effect	Final CSR	30 June 2024
CSL222_3001 / CT-AMT-061-02 A phase 3, open-label, single-dose, multi-center multinational trial investigating a serotype 5 adeno-associated viral vector	<u>Primary objective</u> To demonstrate the non-inferiority of AMT-061 (2 × 10^{13} gc/kg) during the 52 weeks following establishment of stable factor IX expression (months	Long term effect	Final CSR	31 October 2025

Study Status	Summary of objectives	Efficacy uncertainties addressed	Milestones	Due Date
containing the Padua variant of a codon-optimized human factor IX gene (AAV5-hFIX-Padua) administered to adult subjects with severe or moderately severe hemophilia B. Ongoing	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 annualised bleeding rate (ABR). Secondary objective To demonstrate additional efficacy and safety aspects of systemic administration of AMT-061.			
CSL222_4001 An observational post-authorization Long-term Follow-up Study to Characterize the Safety and Effectiveness of HEMGENIX (Etranacogene Dezaparvovec) in Patients with Hemophilia B Planned	Primary Objective To investigate the long-term effectiveness profile in adults with hemophilia B who are treated with HEMGENIX or are on continuous FIX prophylaxis by following them for a period of 15 years. Secondary Objective To characterise the long-term safety in adults with hemophilia B who are treated with HEMGENIX or are on continuous FIX prophylaxis by following them for a period of 15 years.	Effect irrespective of baseline anti-AAV5 NAb titer	Protocol submission 1-year follow-up interim analysis report after the first 50 subjects are enrolled in Study CSL222_4001	31 March 2023 31 December 2026

2.7.3. Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Hepatotoxicity	Routine risk minimisation measures: SmPC sections 4.2, 4.4, 4.8 Legal status: Prescription only product. Additional risk minimisation measures: Health care professional guide, patient guide and patient card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Questionnaire on Liver toxicity Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_5001 • Study CSL222_3003 • Study CSL222_2001 • Study CSL222_3001
Infusion reactions (including hypersensitivity)	Routine risk minimisation measures: SmPC sections 4.2, 4.4, 4.8	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	Legal status: Prescription only product. Additional risk minimisation measures: None	None Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_2001 • Study CSL222_3001
Risk of malignancy in relation to vector integration in the DNA of body cells	Routine risk minimisation measures: SmPC section 4.2, 4.4 Legal status: Prescription only product. Additional risk minimisation measures: Health care professional guide, patient guide and patient card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Questionnaire on Hemgenix Liver malignancy Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_3003 • Study CSL222_5001 • Study CSL222_2001 • Study CSL222_3001
Bleeding as a result of lack of efficacy due to immune-mediated neutralisation of the AAV-5 vector capsid	Routine risk minimisation measures: SmPC sections 4.2, 4.4, 5.1 Legal status: Prescription only product. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_2001 • Study CSL222_3001
Thromboembolic events	Routine risk minimisation measures: SmPC section 4.2., 4.4 Legal status: Prescription only product. Additional risk minimisation measures: Health care professional guide, patient guide and patient card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Questionnaire on Thromboembolic Events (TEE) Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_3003 • Study CSL222_5001 • Study CSL222_2001 • Study CSL222_3001

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Germline transmission	Routine risk minimisation measures: SmPC sections 4.2, 4.4, 4.6 Legal status: Prescription only product. Additional risk minimisation measures: Health care professional guide, patient guide and patient card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_5001 • Study CSL222_2001 • Study CSL222_3001
Transmission to third parties (horizontal transmission)	Routine risk minimisation measures: SmPC sections 4.4, 5.2 Legal status: Prescription only product. Additional risk minimisation measures: Health care professional guide, patient guide and patient card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_5001 • Study CSL222_2001 • Study CSL222_3001
Development of FIX inhibitors	Routine risk minimisation measures: SmPC sections 4.1, 4.2, 4.4, 4.8 Legal status: Prescription only product. Additional risk minimisation measures: Health care professional guide, patient guide and patient card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_3003 • Study CSL222_5001 • Study CSL222_2001 • Study CSL222_3001
Use in patients with severe hepatic impairment	Routine risk minimisation measures: SmPC sections 4.2, 4.3, 4.4, 4.5, 5.2 Legal status: Prescription only product. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Questionnaire on Liver toxicity Additional pharmacovigilance activities: Study CSL222_4001

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Long-term effect	Routine risk minimisation measures: SmPC section 4.2, 4.4 (risk of carcinogenicity) Legal status: Prescription only product. Additional risk minimisation measures: Health care professional guide and patient guide	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: • Study CSL222_4001 • Study CSL222_3003 • Study CSL222_5001 • Study CSL222_2001 • Study CSL222_3001
Use in female patients	Routine risk minimisation measures: SmPC section 4.2, 4.6 (Fertility, pregnancy and lactation) Legal status: Prescription only product. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: Study CSL222_4001

2.7.4. Conclusion

The CAT considers that the risk management plan version 1.0 is acceptable.

The CHMP endorses the CAT conclusion on the RMP as described above.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant requested alignment of the PSUR cycle with the international birth date (IBD). The IBD is 22 November 2022. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

A request for a translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant proposing that the details listed in Article 54 appear in only one official language (English) on all packaging components (vial and outer carton) and the package leaflet. The main ground of the justification was the low estimated number of patients treated per country due to the low incidence/prevalence of the condition in the EU, and the fact that the medicinal product will administered by healthcare professional in a clinical setting.

The QRD The Group partially accepted this translation exemption request. All Member states agreed to have an EN only vial label. For the outer carton, a bi-lingual carton English(EN)/Germany(DE) should be provided. An English only package leaflet was not accepted. The QRD group agreed that a printed package leaflet in English is included inside the secondary packaging (outer carton). However, the applicant should distribute the translated printed package leaflets in the national language alongside the cartons.

2.9.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Hemgenix (etranacogene dezaparvovec) is included in the additional monitoring list as

- It contains a new active substance
- It is a biological product
- It is approved under a conditional marketing authorisation

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Hemgenix is for the treatment of severe and moderately severe Haemophilia B (congenital Factor IX deficiency) in adult patients without a history of Factor IX inhibitors.

3.1.2. Available therapies and unmet medical need

The primary aim of care for patients with haemophilia B is to prevent bleeding. Replacement therapy with exogenous FIX provides a temporary correction of the coagulation factor deficiency by increasing FIX levels and thereby reducing bleeding.

Factor IX Prophylaxis

Prophylaxis with FIX should be considered in all people with severe haemophilia B (including those classified as non-severe according to their basal FIX levels but with a severe bleeding phenotype); in these HB patients, prophylaxis should be initiated as early as possible (i.e. prior to the onset of joint bleeding), and thereafter, treatment should not be interrupted. Both SHL-FIX and EHL-rFIX are effective treatment options for prophylaxis and either SHL-FIX or EHL-FIX products can be used to offer adequate haemostatic cover for bleeds, surgery and invasive procedures. Dose and frequency of prophylactic FIX treatment should be adapted to the clinical phenotype (e.g. bleed rates) and lifestyle considerations, and not based exclusively on plasma trough levels. The current treatment options for haemophilia B have several limitations. Treatment with prophylactic regular IV injections of FIX 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 FIX 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 morbidity and mortality rate.

There is also a risk of developing neutralizing antibodies (nAbs) against the administered FIX. 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 haemophilia can have a substantial effect on mental wellbeing, particularly among young people and signs of major depressive disorder are not uncommon. The economic burden for the society is significant.

There remains an unmet medical need in HB since available treatment options require long-term, chronic treatments with a high degree of compliance to the prescribed treatment schedule to be effective.

3.1.3. Main clinical studies

The clinical study programme consists of four studies. The initial phase 1 study, CT-AMT-060-01, and its long-term extension study CT-AMT-060-04, used the predecessor product AMT-060 and are considered supportive. No CSR is available for the LTE study at the present time. The main evidence for efficacy and safety derives from the phase 2b trial CT-AMT-061-01 and the pivotal trial CT-AMT-061-02, in which a combined 57 subjects were enrolled. Two and a half years of follow-up is available for the phase 2 study, and 1.5 years for the pivotal study.

CT-AMT-061-01 is an ongoing Phase IIb trial consisting of a screening phase, a treatment plus post-treatment follow-up phase, and a long-term follow-up phase. After a maximum 6-week screening period, 3 subjects received a single IV dose of 2 × 1013 gc/kg AMT-061. Subjects were monitored for tolerance to AMT-061 and detection of immediate AEs for 24 hours (overnight stay) after dosing. The dosing of the subjects was separated by a minimum of 14 calendar days to allow for subject safety monitoring and to ensure appropriate action could be taken in case any acute reactions were observed.

CT-AMT-061-02 (Health Outcomes with Padua Gene; Evaluation in Hemophilia B [HOPE B]) is an ongoing open-label, single-dose, multicentre, multinational trial, with a screening phase/period, a lead-

in phase/period, a treatment plus a post-treatment follow-up phase/period, and a long-term follow-up phase/period. During the lead-in phase, which lasted a minimum of 26 weeks, subjects recorded their use of FIX replacement therapy and bleeding episodes in their dedicated e-diary in order to provide a baseline of bleeding event frequency and FIX consumption. Of the 67 subjects who entered the lead-in period, 13 discontinued and 54 subjects were dosed with AMT-061.

3.2. Favourable effects

In the phase 2b study CT-AMT-061-01, mean FIX activity level at Week 6, the time of the primary endpoint read-out, was 30.6 % measured by the one-stage assay. Individual FIX activity levels achieved by each subject at Week 6 were 23.9%, 30.0%, and 37.8%. At Week 52, FIX activity level was 40.8% measured by the one-stage assay. Individual FIX activity levels achieved by each subject at Week 52 were 31.3%, 40.8%, and 50.2%. At Month 36, the mean FIX activity level was 36.90%, uncontaminated samples were available for 2 subjects and demonstrated that FIX activity levels continued to be elevated, at 32.3% and 41.5%, respectively.

The average ABR for the 3 subjects, calculated as the total number of bleeding episodes divided by the time (in years) at risk, was 0.22 over the period of 3 years (36 months) of follow-up. The ABRs for spontaneous and traumatic bleeding episodes over 3 years (36 months) were both 0.11. There were no bleeding episodes between 2.5 and 3 years of follow-up (both bleeding episodes occurred in the first 18 months post-AMT-061 administration). These ABR values are low, but as this trial had no run-in phase specified in the protocol, a comparison to meaningful pre-treatment data is not possible.

In the pivotal trial CT-AMT-061-02, a significant reduction of unadjusted mean ABR could be shown comparing the lead-in period ABR of 4.11 to the post-treatment ABR of 1.08 recorded during months 7 to 18. The pre-specified NI analysis encompassing a comparison of ABR between the lead-in (4.19) and post-treatment (1.51) period estimated from a negative binomial regression model was significant and non-inferiority to FIX prophylaxis could be declared. In addition, the secondary outcome of superiority over FIX prophylaxis could also be shown. 20.4% of subjects reported joint bleeding episodes post-treatment, compared to 59.3% of during lead-in. The number of subjects who did not experience any bleeding event more than doubled during month 7-18 [34/54 (63.0%)] compared to baseline [14/54 (25.9%)]. With the responses to the D120 LoQ, an ABR analysis for months 7-24 after treatment was provided (not adjusted for multiplicity). The unadjusted ABR was 0.99, with the adjusted ABR 1.51 (0.83, 4.76). 27 (50.0%) of subjects reported no bleeding episode from month 7-24. 27.8% of subjects reported joint bleeds between month 7-24.

FIX activity levels showed clinically relevant values at month 6 (mean 38.95; median 37.30), continued to increase until month 12 (mean 41.48; median 39.90) and then declined slightly until month 18 (mean 36.90; median 35.55) and remained steady at month 24 (mean 36.66; median 33.85). No subject recorded values >150%. External factor IX consumption as well as external FIX infusion rate in the post-treatment period declined to approximately 3% of the value observed during the lead-in period. Fifty-two of 54 subjects remained free from FIX replacement therapy during the follow-up period of 24 months. One of the two subjects who had to return to FIX replacement therapy received only about 10% of the intended dose of AMT-061 due to hypersensitivity and the second had a high anti-AAV5 nAb titre at baseline and did not respond to treatment with Hemgenix.

In supportive study CT-AMT-060-01, the mean endogenous FIX activity levels in Cohort 1 and Cohort 2 ranged from 2.8% to 8.2% and 4.0% to 10.7% of normal based upon the one-stage (aPTT-based) FIX assay, respectively, and remained stable during the post-tapering period (i.e., after discontinuation of FIX prophylaxis post-AMT-060 administration) up to 5 years.

3.3. Uncertainties and limitations about favourable effects

The generalizability of the efficacy results was questioned due to a low number of patients in this single pivotal study (n= 54) together with the fact that 19% of the patients in the lead-in period did not continue into the actual study.

One subject with a nAb titre >3000 at baseline was found to be a non-responder to treatment with AMT-061, while all other twenty subjects who exhibited anti-AAV5 nAbs at baseline developed clinically relevant FIX activity levels and could terminate prophylaxis with exogenous FIX products. These subjects were found to have titres up to 678.2 at baseline, and apart from increased FIX activity they also reported a statistically significantly reduced annualised bleeding rate compared to the lead-in period using their usual FIX prophylaxis. In the context of the conditional marketing authorisation, the applicant has committed to conduct further investigation of the effectiveness of Hemgenix regardless of the preexisting anti-AAV5 nAb titre in a post-authorisation efficacy study (PAES).

Some decline of FIX activity could be noticed during the 18 months of follow-up available in the submitted interim CSR. Some subgroups achieved appreciably lower levels of FIX activity, therefore this decline could lead to the need for a return to factor replacement in the near future. In order to further elucidate the durability of the response, the applicant committed in the context of the conditional marketing authorisation to submit the final study results, including 5 years' follow-up, of studies CT-AMT-061-01 and CT-AMT-061-02. During long-term monitoring post marketing, safety and efficacy parameters will be collected up to 15 years and increase the understanding of the durability of the achieved FIX activity.

3.4. Unfavourable effects

All participants in the ISS Safety Population (n=57) experienced at least 1 adverse event and a total number of 613 AEs were reported. The majority of events were mild (incidence 98.2%, 460 AEs) or moderate (incidence 68.4%, 135 AEs) in severity. Severe events were reported by 11 subjects (19.3%, 18 AEs).

Based on the assessment of the Investigator, 95 treatment-related AEs were reported for 39 participants (68.4%). The most frequent related AEs were ALT increased (9 subjects [15.8%] with 10 AEs), Headache (9 subjects [15.8%] with 10 AEs), Influenza-like illness (7 subjects [12.3%] with 8 AEs), AST increased (5 subjects [8.8%] with 6 AEs), Blood Creatine Phosphokinase increased (4 subjects [7%] with 6 AEs), Dizziness, Fatigue, Nausea (each by 4 subjects [7%] with 4 AEs). The reported treatment-related TEAEs were mostly mild (27 subjects [47.4%]) or moderate (11 subjects [19.3%]). One subject (1.8%) reported two severe treatment-related events (ALT and AST increased).

TEAEs related to IP administration: Seven subjects experienced TEAEs Qualifying for Special Notification related to IP administration; i.e. Infusion Related Reaction (2 [3.5%]), Hypersensitivity (1 [1.8%]), Infusion Site reaction (1 [1.8%]), Dizziness (2 [3.5%]), Eye pruritus (1 [1.8%]), Flushing (1 [1.8%]), Headache (1 [1.8%]), Abdominal Pain Upper (1 [1.8%]), Urticaria (1 [1.8%]), Chest Discomfort (1 [1.8%]), and Pyrexia (1 [1.8%]). One of the 7 subjects had a TEAE of Hypersensitivity during administration of etranacogene dezaparvovec and resulted in discontinuation of treatment and receipt of a partial dose (approximately 10%). Three subjects with infusion reactions required a dose interruption. The infusions resumed at a lower infusion rate with or without additional treatment with corticosteroids and/or antihistamines. Section 4.4 of the SmPC already includes a warning statement in this regard.

TEAEs by Anti-AAV5 nAb Status at Baseline: While a slightly higher rate of AEs per subject was observed in participants who were seropositive for anti-AAV5 nAbs (mean 12 AEs per subject vs. mean

9.9 AEs per subject), there was no difference regarding treatment-related AEs (mean 1.6 per subject vs. 1.7 AEs per subject). Five of the 7 subjects who reported TEAEs related to IP administration were positive for anti-AAV5 nAbs at baseline. The incidence of SAEs was higher in the subgroup of participants who were seropositive at baseline (37.5%, 10 SAEs in 26 subjects), compared to participants who were seronegative (15.2%, 5 SAEs in 33 subjects). Based on the evaluation of the type of reported SAEs, it seems that positive anti-AAV5 NAb status at baseline does not raise safety concerns with etranacogene dezaparvovec treatment itself but may contribute to the need of remaining on prophylaxis with FIX replacement therapy.

Hepatotoxicity: Nine subjects (15.8% of the ISS Population) used systemic corticosteroids for transaminase elevations after administration of AMT-061. The mean corticosteroid treatment duration for those subjects was 79.8 days [range 51 to 130 days]. These nine subjects received steroids as treatment for the liver enzyme elevations of either > ULN (n = 8) or > 2 × baseline value (n = 1), including prednisone, prednisolone, and methylprednisolone. All transaminase elevations that were treated with steroids had an onset within 3 months post dose, with the earliest onset at Week 3. All subjects discontinued steroid use before Week 26. All TEAEs regarding elevated transaminase were non-serious and resolved. One subject in study CT-AMT-061-01 had moderate TEAEs of ALT increased, AST increased, and blood creatine phosphokinase increased between Days 787 and 806 that resolved without treatment. No additional immunosuppressive treatment (other than corticosteroids) was necessary for any patient.

Serious Adverse Events and deaths: In the ISS Population, 15 (26.3%) subjects experienced 18 treatment-emergent SAEs. Of the 18 SAEs, 2 were mild (grade 1) in severity, 8 were moderate (grade 2), and 8 were considered as severe (grade 3). No SAE was considered related to treatment.

3.5. Uncertainties and limitations about unfavourable effects

The evaluation of the safety profile of Hemgenix is challenging due to multiple factors: lack of a control arm, small sample size, limited data on long term follow-up, and potential consequences of non-clinical and clinical findings related to integration of AAV.

Rare/uncommon adverse events: The sample size is too small to detect rare or uncommon adverse events. Even some common events may have not been detected.

Vector integration, potential carcinogenicity and long-term safety: Vector integration was observed in nonclinical studies with mice and cynomolgus macaques. The integration site analysis study performed on liver biopsies of mice and cynomolgus monkeys showed low level of vector integration. While recombinant AAV are not expected to integrate their genome in host cells at high frequency, all integration events could potentially contribute to tumoral transformation.

Vector integration confirmed by human liver biopsies from one participant of the clinical trials. Considering the lack of data in other patients (no other human biopsies available), detailed estimations regarding vector integration cannot be made.

One case of hepatocellular carcinoma was observed during Study CT-AMT-061-02. The event was considered unlikely related by the Investigator with support from an external expert group.

Considering the small sample size and the limited follow-up duration, the theoretical risk of malignancy due to vector integration cannot be estimated, which remains an uncertainty for the B/R assessment.

Hepatotoxicity: Some patients required corticosteroid treatment for elevated transaminases. All participants were tapered off within the first half year. Until the data cut-off, no participant

experienced reoccurrence of elevated transaminases. However, due to the limited data, a potential reoccurrence of elevated transaminases requiring treatment cannot be excluded.

Concomitant hepatotoxic medications: There is lack of data regarding the concomitant use of hepatotoxic agents.

Use in patients with liver impairment or active infections: Patients with advanced fibrosis, other hepatic disorders like uncontrolled HIV or active hepatitis B/C infections were excluded from the clinical trials. Due to the safety profile (elevated transaminases, potential risk of carcinogenicity) and the fact that the liver is the target organ for this gene therapy, section 4.3 of the SmPC includes contraindications for patients with active infections (either acute or uncontrolled chronic) and for patients with known *advanced* hepatic fibrosis, or cirrhosis.

Patients seropositive for anti-AAV5 nAbs prior to treatment: While no meaningful differences between seropositive and seronegative patients were noted regarding the overall profile of adverse events, the (limited) clinical data suggest a trend for an increased risk for infusion reactions (including hypersensitivity) in patients who are seropositive prior to treatment. The majority of participants who reported TEAEs related to IP administration were seropositive, including the subject who discontinued due to a hypersensitivity event. The risk of infusion-related reactions will be further characterised in the post-marketing setting.

Lack of data in patient subgroups: The clinical data in subgroups such as elderly, subjects of different ethnicities, or HIV-positive patients is limited. No female haemophilia B patients were recruited.

Vector shedding and horizontal/vertical transmission: In the absence of respective assays, infectivity of the shed material has to be assumed, according to the ICH guideline (EMEA/CHMP/ICH/449035/2009). Median time to vector shedding negative in semen was 45.8 weeks (95% CI 34.1, 52.1 weeks) and samples from 9 subjects were still positive for vector DNA at or after day 182. Based on the available clinical data, a recommendation for barrier contraception for 12 months was included in section 4.6 of the SmPC.

3.6. Effects Table

Table 28. Effects Table for Hemgenix (data cut-off: 25 January 2022)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects			**Months 7-18**	**Lead-in**		
ABR	Annualised Bleeding rate	Bleeds/ year	1.51	4,19	Comparison with lead-in period of at least 26 weeks during which subjects recorded their bleeding events and FIX use; N=54	Study CT-061-02
Median FIX activity level	Endogenous FIX activity	% of normal	37.30 Month 6 39.90 Month 12 33.55 Month 18	1		
Consumption of exogenous FIX (Mean)	Use of factor replacement	IU/year	8399.1	257,338.8		
Unfavourable Effects						
AEs SAEs	Incidence Incidence	% %	100 24.6	N/A	ISS Population (n=57)	Studies CT-061-01 and CT-061-01 (ISS Population)
Hepatocelluar carcinoma	Incidence	%	1.8	N/A	Once case, considered not related, but remaining questions	
SOC Neoplasms	Incidence	%	10.5	N/A	11 AEs in 6 subjects, none was considered related, but narratives requested	
Treatment-related AEs: ALT increased AST increased	 Incidence Incidence	 % %	 15.8 8.8	N/A	Corticosteroid treatment of elevated transaminases in 9 subjects	
FIX inhibitor			None	N/A		
Non-clinical and clinical integration of AAV			Reported in animals and biopsies from a trial participant	N/A		

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The submitted clinical efficacy data show a statistically significant and clinically relevant reduction of bleeding frequency, i.e from 4.19 at baseline to 1.51 post-treatment in subjects treated with

Hemgenix. The ABR recorded during post-treatment months 7-18 was compared to the same subjects' bleeding frequency during the lead-in period of at least 6 months, where the standard of care, prophylactic FIX replacement, was administered.

Endogenous FIX activity achieved clinically relevant levels, i.e. a median of 39.9% at month 12 in the majority (52/54) of subjects, with no subject showing supraphysiologic FIX activity. Use of exogenous FIX as well as FIX infusion rate fell to approximately 3% of values reported during lead-in.

Updated efficacy data from 24 months of follow-up from pivotal trial AMT-061-02 and 36 months of follow-up from trial AMT-061-01 continue to show satisfactory outcomes with regard to clinically relevant FIX activity (mean 36.66; median 33.85) and a sustained low ABR of 0.99 (adjusted 1.51; CI:0.83, 4.76).

The durability of the therapeutic effect has been shown to be stable up until 24 months of follow-up in the pivotal trial. In order to further elucidate the durability of the response, the applicant committed in the context of the conditional marketing authorisation to submit the final study results, including 5 years' follow-up, of studies CT-AMT-061-01 and CT-AMT-061-02. During long-term monitoring post marketing, safety and efficacy parameters will be collected up to 15 years and increase the understanding of the durability of the achieved FIX activity.

Subjects were enrolled into the pivotal study irrespective of their pre-existing anti-AAV nAb titre. Twenty subjects were found to have titres up to 1:678.2 at baseline, and 33 subjects were negative. While overall a numerically lower mean Factor IX activity was observed in patients with pre-existing neutralising anti-AAV5 antibodies, no clinically meaningful correlation was identified between patients` pre-existing anti-AAV5 antibody titre and their factor IX activity at 18 months post-dose. One patient with a titre of 1:3212 at screening did not respond to etranacogene dezaparvovec treatment, with no factor IX expression and activity. Further investigation of the effectiveness of Hemgenix will be conducted in a post-authorisation efficacy study regardless of the pre-existing anti-AAV5 nAb titre.

Based on the limited short to medium-term safety data, gene therapy with AMT-061 was relatively well tolerated by the majority of study participants. The majority of reported adverse events were mild or moderate in severity.

The most significant short-term safety concerns are potential infusion related (and hypersensitivity) reactions. One administration was discontinued and for some patients the infusion was temporarily paused and resumed at a reduced infusion rate after treatment with antihistamines and/or corticosteroids. Considering that only one administration of AMT-061 is necessary, the risk of hypersensitivity appears manageable.

The most relevant medium-term safety concern seems to be the risk of experiencing elevated liver enzymes (ALT, AST), which may necessitate treatment with corticosteroids in some recipients of AMT-061. Intake of corticosteroids over an extended period of time poses its own risk of developing adverse events. Of note, a nonclinical study suggests that liver injury increases the risk of HCC in mice who received AAV gene therapy (Dalwadi et al. 2020).

Vector integration was shown in mice and cynomolgus monkeys, and has been confirmed by biopsies from a trial participant who developed a hepatocellular carcinoma during the clinical trial. This may indicate a long-lasting, potentially life-long risk of malignancy as a result of vector integration. It is very likely that the significant uncertainty regarding carcinogenicity cannot be resolved in the near future, since the follow-up duration during the trials is too short and the sample size too small.

The sample size of the presented clinical trials is too small to detect rare or uncommon adverse events. Even some common events may not have been detected. This would need to be addressed by post marketing surveillance.

3.7.2. Balance of benefits and risks

The short- to medium-term magnitude and durability of the demonstrated clinical benefits (i.e. clinically relevant levels of endogenous FIX activity, improvement of bleeding frequency over standard of care, minimal need for external factor replacement) of treatment with Hemgenix are considered to outweigh the observed short- to medium-term safety concerns (i.e. infusion reactions, influenza-like illness, headache, transaminitis).

There are still uncertainties with regard to the durability of the effect and long-term safety (i.e. hepatic safety, risk of malignancy as a result of vector integration), which have to be addressed in the context of the CMA and by the multi-year follow-up investigations as requested in the Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products (EMEA/149995/2008 rev.1).

Overall, the Benefit/Risk is considered positive.

3.7.3. Additional considerations on the benefit-risk balance

Comprehensive efficacy and safety data are lacking in the current MAA.

The sample size of 57 patients is considered very small. While the two years of follow-up provided show that the expression of FIX activity appears to be stable over this duration, the long-term durability of the treatment effect and long-term safety are still unknown factors.

Furthermore, uncertainties remain regarding the impact of neutralizing anti-AAV capsid antibodies on efficacy and safety and these cannot be comprehensively characterised based on the limited available data.

Conditional marketing authorisation

As comprehensive data on the product are not available, a conditional marketing authorisation was proposed by the CAT during the assessment and agreed by the applicant.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it aims at the treatment of a seriously debilitating disease. In addition, the product is designated as an orphan medicinal product.

Furthermore, the CAT considers that the product fulfils the requirements for a conditional marketing authorisation:

- The benefit-risk balance is positive, as discussed.
- It is likely that the applicant will be able to provide comprehensive data coming from the ongoing studies CT-AMT-061-01 and CT-AMT-061-02 and planned study CSL222_4001. The present limitations of the dataset will be addressed with three specific obligations intended to provide additional data on the durability of the effect and long-term safety (final CSRs of the phase II and phase III trials, SOB 1 and SOB 2) and efficacy in subjects with haemophilia B irrespective of baseline anti-AAV5 nAb titre (an interim analysis of the PAES CSL222_4001 after the first 50 subjects have reached one year of follow-up; SOB 3). The data expected from these specific obligations will increase the available follow-up to 5 years, at present 24 months follow-up is available for 54 subjects and 36 months for 3 subjects. The 50 subjects to be included in the interim clinical study report of study CSL222_4001 will approximately double the available study population and are anticipated to allow further insights into efficacy outcomes across a broader range of baseline nAb titres.

- Unmet medical needs will be addressed, as Hemgenix requires a single intravenous administration that could free severe and moderately sever haemophilia B patients from therapeutic burden for at least 2 years while the available treatment options require a variable number of injections, i.e. frequent prophylactic infusion of exogenous FIX, or episodically at the time of a bleeding event.
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. As benefit-risk balance on basis of the current data is regarded positive, an additional therapy option for severe haemophilia patients is considered beneficial.

The CHMP endorses the CAT conclusion on conditional marketing authorisation as described above.

3.8. Conclusions

The overall benefit/risk balance of Hemgenix is positive, subject to the conditions stated in section 'Recommendations'.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described above

4. Recommendations

Similarity with authorised orphan medicinal products

The CAT by consensus is of the opinion that Hemgenix is not similar to Alprolix and Idelvion within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000.

The CHMP endorses the CAT conclusion on similarity as described above.

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the benefit- risk balance of Hemgenix is favourable in the following indication:

Hemgenix is indicated for the treatment of severe and moderately severe haemophilia B (congenital factor IX deficiency) in adult patients without a history of factor IX inhibitors.

The CAT therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to special and restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Prior to launch of Hemgenix in each Member State, the marketing authorisation holder (MAH) must agree about the content and format of the educational programme with the National Competent Authorities.

The MAH shall ensure that in each Member State where Hemgenix is marketed, all healthcare professionals and patients/carers who are expected to prescribe, use or oversee the administration of Hemgenix have access to/are provided with the following educational packages. These packages will be translated in the local language to ensure understanding of proposed mitigation measures by physicians and patients:

- Physician Educational Material
- Patient Information Pack.

The Physician Educational Material consists of:

- Guide for Healthcare Professionals;
- The Summary of Product Characteristics;
- The Patient/Care-giver guide;
- The Patient Card.

The Patient Information Pack consists of:

- The Patient/Care-giver guide;
- The Patient Card;
- The patient information leaflet.

The Guide for Healthcare Professionals key messages:

- To inform the patient of the important identified risk of hepatotoxicity and the important potential risks of horizontal and germline transmission, development of Factor IX inhibitors, malignancy in relation to vector genome integration, and thromboembolism, and details on how these risks can be minimised.
- Before a treatment decision is made, the healthcare professional should discuss the risks, benefits, and uncertainties of Hemgenix with the patient when presenting Hemgenix as a treatment option, including:

- That Hemgenix use will require in some cases administration of corticosteroids to manage the liver damage that this medicinal product might induce. This requires adequate monitoring of patients' liver function and avoidance of concomitant use of hepatotoxic medication or agents, to minimise the risk of hepatoxicity and a potential reduced therapeutic effect of Hemgenix.
- That high preexisting neutralising anti-AAV5 antibodies may reduce the efficacy of Hemgenix therapy; patients should be assessed for the titre of preexisting neutralising anti-AAV5 antibodies before Hemgenix treatment.
- That there is a possibility of not responding to treatment with Hemgenix. Patients who do not respond are still exposed to long-term risks.
- That the long-term treatment effect cannot be predicted.
- That there would be no plans to re-administer the medicinal product for patients who do not respond or have lost the response.
- That the patients should be tested for Factor IX inhibitors to monitor development of Factor IX inhibitors.
- Reminding patients about the importance to enroll in a registry for follow up of long-term effects.
- The healthcare professional should provide the patient guide and patient card to the patient.

The Patient/Care-giver Guide key messages:

- Importance to fully understand the benefits and risks of Hemgenix treatment, what is known and not yet known about the long-term effects, related to both safety and efficacy.
- Therefore, before a decision is made about starting on the therapy the doctor will discuss with the patient the following:
  - That Hemgenix will, in some cases, require treatment with corticosteroids to overcome the liver damage that this medicine may produce, and that the doctor will ensure that patients are available for regular blood tests to check response to Hemgenix and assess liver health. Patients should inform the healthcare professional about current use of corticosteroids or other immunosuppressants. If the patient cannot take corticosteroids, the doctor may recommend alternative medicines to manage problems with the liver.
  - That high preexisting immunity against the vector may reduce the efficacy of Hemgenix therapy; patients are expected to be assessed for the titre of preexisting neutralising anti-AAV5 antibodies before the Hemgenix treatment.
  - That not all patients may benefit from treatment with Hemgenix. Patients not responding to treatment are still be exposed to long-term risks.
  - Details how the important potential risks of horizontal and germline transmission, development of Factor IX inhibitors, malignancy in relation to vector genome integration, and thromboembolism can be recognised and minimised by regular monitoring as recommended by doctors, including that:

- The patient should seek immediate medical advice for any symptoms suggestive of a thromboembolic event.
- Male patients of reproductive potential or their female partners should use barrier contraception for one year after administration of Hemgenix.
- That Hemgenix has a viral vector component, and it may be associated with an increased risk of malignant tumour. Regular liver monitoring for at least 5 years after Hemgenix treatment is needed in patients with preexisting risk factors for hepatocellular carcinoma.
- Patients should not donate blood, semen, or organs, tissues, and cells for transplantation
    - That the patient will get a patient card that should be shown to any doctor or a nurse whenever the patient has a medical appointment.
    - The importance to participate in the patients' registry for long-term surveillance of 15 years.

The Patient Card key messages:

- This card is to inform healthcare professionals that the patient has received Hemgenix for haemophilia B.
- The patient should show the patient card to a doctor or a nurse whenever they have an appointment.
- The patient should seek medical advice for any symptoms suggestive of a thromboembolic event.
- The patient should have regular blood tests and examinations as directed by their doctor.
- The card should warn healthcare professionals that the patient may undergo treatment with corticosteroids for minimising the risk of hepatotoxicity with Hemgenix.

The CHMP endorses the CAT conclusion on the additional risk minimisation measures.

- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
In order to further characterise the long-term efficacy and safety of etranacogene dezaparvovec in adult patients with severe and moderately severe haemophilia B (congenital Factor IX deficiency) without a history of factor IX inhibitors, the MAH should submit the final analysis report of a study from a registry, according to an agreed protocol.	31 December 2044

The CHMP endorses the CAT conclusion on the obligation to conduct post-authorisation measures as

described above.

Specific obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
In order to confirm the efficacy and safety of etranacogene dezaparvovec in adult patients with severe and moderately severe Haemophilia B (congenital Factor IX deficiency) without a history of Factor IX inhibitors, the MAH should submit the final results including 5 years follow-up of the pivotal Study CT-AMT-061-01.	30 June 2024
In order to confirm the efficacy and safety of etranacogene dezaparvovec in adult patients with severe and moderately severe Haemophilia B (congenital Factor IX deficiency) without a history of Factor IX inhibitors, the MAH should submit the final results (5 years of data) of pivotal Study CT-AMT-061-02 with 54 subjects.	31 October 2025
In order to confirm the efficacy and safety of etranacogene dezaparvovec in adult patients with severe and moderately severe Haemophilia B (congenital Factor IX deficiency) without a history of Factor IX inhibitors, irrespective of baseline anti-AAV5 neutralising antibody titre, the MAH should submit the 1-year follow-up interim analysis report after the first 50 subjects are enrolled in Study CSL222_4001.	31 December 2026

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

These conditions fully reflect the advice received from the PRAC.

The CHMP endorses the CAT conclusion on the conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

New active substance status

Based on the review of available data on the active substance, the CAT considers that etranacogene dezaparvovec is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

The CHMP endorses the CAT conclusion on the new active substance status claim.