Taiwan Food and Drug Administration

And

Center for Drug Evaluation

REVIEW REPORT

Trade Name：恆傑凝注射劑/ Hemgenix

Active Ingredient：Etranacogene dezaparvovec

Applicant：傑特貝林有限公司

Dosage Form & Strengths：注射液劑，$1x10^{13}$ 基因體拷貝/毫升 / Injections, $1x10^{13}$ genome copies (gc)/mL

Indication：適用於治療年齡18歲以上且須使用第九凝血因子預防性療法的中重度至重度B型血友病病人，必須沒有第九凝血因子抑制因子病史，且既有第五血清型腺相關病毒(AAV5)中和抗體效價低於1:900。

Hemgenix is indicated for the treatment of adults aged 18 and older with severe and moderately severe Hemophilia B requiring Factor IX prophylaxis therapy, without a history of Factor IX inhibitors, and with pre-existing neutralizing antibodies to adeno- associated virus serotype 5(AAV5) titer below 1:900.

License Number：MOHW-BI 001269

Approval Date：2024/12/20

Table of Content

1. Executive Summary

1.1. Background

Etranacogene dezaparvovec is a recombinant adeno-associated virus serotype 5 (AAV5) vector containing an expression cassette encoding a codon-optimized DNA sequence for the Padua-variant of the human factor IX (hFIXco-Padua [R338L]) under the control of a liver-specific promoter. It is designed for the long-term expression of functional human factor IX (FIX) in the liver for the long-term treatment of hemophilia B.

The proposed indication of this New Drug Application (NDA) is:

Etranacogene dezaparvovec 為基因療法，以腺相關病毒為載體，已核准用於患有 B 型血友病(先天缺乏第九凝血因子)，且既有 AAV5 中和抗體效價低於 1:900 的成人病人，以降低出血事件頻率，及滿足對第九凝血因子補充療法的需求，病人應： ● 目前使用第九凝血因子預防性療法， ● 或目前或過去曾發生有生命危險的出血，或重複、嚴重自發性出血事件。

The proposed posology is:

僅供單劑靜脈輸注使用。 Etranacogene dezaparvovec 的劑量為單劑每公斤(kg)體重(bw) 2×10^{13} 個基因體拷貝(gc)。

1.2. Chemistry, Manufacturing and Controls (CMC) Summary

The stability results supported the shelf life of the drug product protected from light at 5 ± 3℃ for 18 months.

1.3. Non-clinical Pharmacology/Toxicology Summary

Overall, the non-clinical program done to support the NDA for etranacogene dezaparvovec is considered acceptable. The non-clinical information in the proposed labeling is generally adequate.

1.4. Pharmacokinetics/Pharmacodynamics Summary

Clinically relevant and statistically significant increase in FIX activity were observed after etranacogene dezaparvovec infusion in clinical studies.

No dedicated hepatic impairment study was conducted. Subjects with alanine aminotransferase (ALT) elevation after etranacogene dezaparvovec infusion had lower mean FIX activity compared to subjects without ALT elevation after etranacogene dezaparvovec infusion.

No dedicated renal impairment study was conducted. However, the FIX activity was comparable among subjects with normal function (CrCl: ≥ 90 mL/min) and limited subjects with mild (CrCl: 60 – 89 mL/min, n = 7) or moderate (CrCl: 30 – 59 mL/min, n = 1) renal impairment.

No drug-drug interaction studies had been performed.

Based on shedding study, the time to reach a negative shedding result was longest in blood, followed by in semen, feces, saliva, nasal secretions and urine. In Study CT-AMT-061-02, 46/54 (85.2%) subjects attained the status of no longer shedding vector DNA from blood at the time of the 3-year database extract. The median time of absence of shedding from blood was 52.6 (95% confidence interval [CI]: 48.1, 77.9) weeks. Forty-five (45/54, 83.3%) subjects attained the status of no longer shedding vector DNA from semen at the time of the 3-year database extract. The median time of absence of shedding from semen was 43.7 (95% CI: 34.1, 51.9) weeks.

The exact race (East Asian) effect on the pharmacokinetic and pharmacodynamic of etranacogene dezaparvovec can not be concluded due to lack of the information about race and ethnicity. However, the ethnic difference can be considered negligible because of the nature of gene products.

Overall, the pharmacokinetic studies conducted were satisfactory met the minimum requirement to support this NDA.

1.5. Clinical and Statistical Efficacy Summary

There were two clinical studies to support the efficacy of proposed indication.

1.5.1. Study CT-AMT-061-01

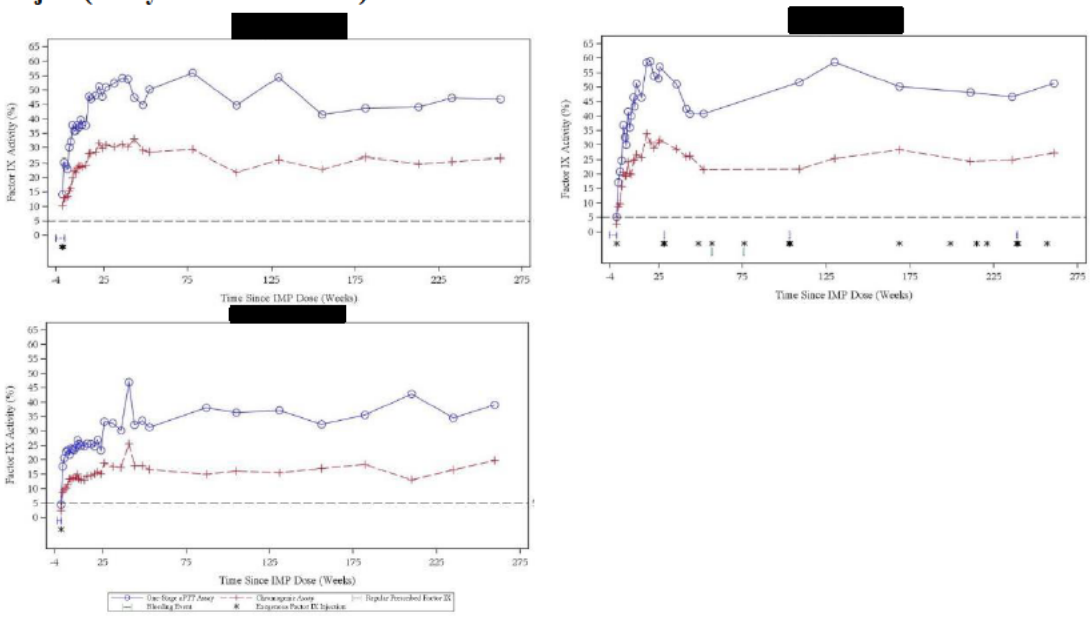
Study CT-AMT-061-01 was a multicenter, open-label, single-arm study in adult male subjects with moderately severe to severe (FIX:C ≤ 2%) hemophilia B. Subjects with FIX inhibitor were excluded.

Three subjects were enrolled and treated with etranacogene dezaparvovec. Two subjects had severe hemophilia B and 1 subject had moderately severe hemophilia B. In the 12 months prior to screening, all subjects used prophylactic FIX replacement therapy and had 1, 3 or 5 bleeding spontaneous bleeding episodes, respectively. All 3 subjects had a prior hepatitis C infection and 2 subjects had a controlled human immunodeficiency virus (HIV) infection. All subjects had pre-existing neutralizing antibody to AAV5 and titers were 19.5, 22.1 and 33.0 at baseline prior to etranacogene dezaparvovec infusion. All 3 subjects completed five years of follow-up.

After etranacogene dezaparvovec infusion, all 3 subjects had increased FIX activity and maintained up to Month 60 (Figure 1). One subject had 2 bleeding episodes (1 spontaneous and 1 traumatic). The annualized bleeding rate (ABR) over 5 years of

follow-up was 0.14. Only 1 subject received exogenous FIX products for on-demand use, surgeries or procedures.

Figure 1 Factor IX Activity (%), Exogenous Factor IX Use, and Bleeding Episodes Over Time by Subject (Study CT-AMT-061-01)



Abbreviations: aPTT = activated partial thromboplastin time; FIX = factor IX.
"Contaminated" results were defined to be a value from a blood sample obtained within 5 half-lives of peevious FIX therapy; exogenous FIX injections are shown in the graphs above.
Factor IX activity was not available at Month 18 (study visit not conducted) and Month 42 (study visit not conducted) and was contaminated at Month 36 (study visit conducted out of window on Day 1182 [Week 168]) for Subject [redacted] see

1.5.2. Study CT-AMT-061-02

Study CT-AMT-061-02 is an ongoing, multinational, multicenter, open-label, single-arm study in adult male subjects with moderately severe to severe (FIX:C $\leq$ 2%) hemophilia B. Subjects with FIX inhibitor were excluded.

Eligible subjects recorded their use of FIX replacement therapy and bleeding episodes in the e-diary during the lead-in phase, which lasted a minimum of 26 weeks. After that, subjects received etranacogene dezaparvovec infusion and followed for totally 5 years.

A total of 54 subjects received etranacogene dezaparvovec infusion: 53 subjects completed treatment and 1 subject received a partial dose (10%) due to a treatment-emergent adverse event (TEAE) of hypersensitivity. The mean (standard deviation [SD]) and median (range) age of these subjects were 41.5 (15.8) and 37.0 (19 – 75) years. Most subjects were White (74.1%) and had severe hemophilia B (81.5%). All subjects used prophylactic FIX therapy. Three (5.6%) subjects were HIV positive. There were 9 (16.7%) and 31 (57.4%) subjects with a history of hepatitis B and hepatitis C, respectively. Subjects who were currently receiving antiviral therapy for hepatitis B or C, positive for hepatitis B surface antigen (except this was due to a previous hepatitis B vaccination rather than active hepatitis B infection), positive for hepatitis B virus DNA or positive for hepatitis C virus RNA were not enrolled.

Twenty-one subjects had pre-existing neutralizing antibody to AAV5 and titers ranged from 8.5 to 678.2 for 20 subjects and 3212.3 for the remaining 1 subject.

The mean adjusted ABR for all bleeding episodes was reduced from 4.19 (95% CI: 3.22, 5.45) in the lead-in phase to 1.51 (95% CI: 0.81, 2.82) for the Month 7 to 18 post-treatment period. The adjusted ABR rate ratio for the Month 7 to 18 post-treatment period to lead-in period was 0.36 (95% Wald CI: 0.20, 0.64). Thirty-four (63.0%) subjects did not have bleeding episode during the Month 7 to 18 post-treatment period compared to 14 (25.9%) subjects in the lead-in phase. Both non-inferiority and superiority of etranacogene dezaparvovec over standard of care were achieved. Consistent results were observed during the Month 7 to Month 24 post-treatment period and Month 7 to 36 post-treatment period (Based on Year 3 Summary Report).

Consistent results were observed for bleeding subtypes.

The mean adjusted ABR for all FIX-treated bleeding episodes was reduced from 3.65 (95% CI: 2.82, 4.74) in the lead-in phase to 0.84 (95% CI: 0.41, 1.73) for the Month 7 to 18 post-treatment period and 0.99 (95% CI: 0.48, 2.03) for Month 7 to 24 post-treatment period.

FIX activity increased and were generally maintained after etranacogene dezaparvovec infusion. Most subjects achieved FIX activity > 5% [redacted] Two subjects failed to express endogenous human FIX: one subject with a high baseline neutralizing antibody to AAV5 and the other received a partial dose due to AE.

Except the two subjects who failed to express endogenous human FIX, the remaining 52 subjects discontinued and remained free of routine FIX prophylaxis. In the updated summary report, another subject with low initial FIX activity lost FIX expression between Month 29 and 30 after treatment, had increased number of hemorrhage and returned to routine FIX prophylaxis.

The infusions and consumption of FIX products decreased following etranacogene dezaparvovec infusion.

1.6. Clinical Safety Summary

Fifty-seven subjects received etranacogene dezaparvovec infusion in the two clinical studies, and 54 subjects had been followed for more than 2 years after infusion.

All subjects experienced at least one TEAE. The most commonly reported TEAEs included arthralgia, headache, nasopharyngitis, fatigue, ALT increased, back pain, COVID-19, pain in extremity, aspartate aminotransferase (AST) increased, blood creatine phosphokinase increased, influenza-like illness, oropharyngeal pain, toothache, hypertension, cough, diarrhea, and nausea.

Eleven (19.3%) subjects experienced 18 severe TEAE and 15 (26.3%) subjects experienced 18 serious TEAEs. Blood loss anemia was the only severe or serious TEAE reported for 2 subjects, the remaining events were reported for 1 subject. Of these events, the severe TEAEs of ALT increased and AST increased in 1 subject were considered related to study treatment.

There was one fatal event of cardiogenic shock. This event was considered unrelated to study treatment.

Fourteen (24.6%) subjects experienced treatment emergent ALT or AST increased, and 9 subjects (15.8%) received steroid treatment for these events of either > upper limit of normal (n = 8) or > 2 × baseline values (n =1). No subject met the definition of drug-induced liver injury (DILI). All events were resolved. The incidence of ALT or AST increased was similar between subjects with or without baseline anti-AAV5 neutralizing antibodies.

Twelve (21.1%) subjects had at least one TEAE qualified for special notification according to predefined criteria. One subject had hepatocellular carcinoma. This event was considered unrelated to study treatment based on pre-existing risk factors, genetic studies and independent external experts review. Seven (12.3%) subjects had TEAEs qualified for special notification related to etranacogene dezaparvovec infusion. The incidence of these events were higher in subjects with anti-AAV5 neutralizing antibodies at baseline (5/24, 20.8%) compared to subjects without anti-AAV5 neutralizing antibodies at baseline (2/33, 6.1%).

All 57 subjects were negative at baseline for FIX inhibitors and remained negative through to Month 24 after etranacogene dezaparvovec infusion.

1.7. Conclusion and Recommendation

Based on the above review, the pre-clinical and clinical data of etranacogene dezaparvovec for the treatment of moderately severe to severe hemophilia B is acceptable. Subjects with moderately severe to severe hemophilia B had increased FIX activity and decreased ABR after etranacogene dezaparvovec infusion.

The main issue related to this NDA is anti-AAV5 neutralizing antibodies. Theoretically, these neutralizing antibodies may impede the transduction, decrease the

expression of FIX and FIX activity leading to treatment failure. However, subjects with baseline anti-AAV5 neutralizing antibodies were enrolled in the clinical studies of etranacogene dezaparvovec.

Except one subject with extreme high baseline anti-AAV5 neutralizing antibody, the remaining 20 subjects with baseline anti-AAV5 neutralizing antibody still had decreased ABR after etranacogene dezaparvovec infusion, although the FIX activity might be numerically lower compared to those without baseline anti-AAV5 neutralizing antibody. The proportion of these subjects achieving FIX activity > 40 was also lower compared to subjects without baseline anti-AAV5 neutralizing antibody. Besides, the majority of these subjects still maintained FIX activity > 5% through to Month 24 after etranacogene dezaparvovec infusion (Figure 3).

The safety profiles between subjects with or without baseline anti-AAV5 neutralizing antibody were generally comparable. TEAEs related to etranacogene dezaparvovec infusion were more commonly reported in subjects with baseline anti- AAV5 neutralizing antibody, but the majority of events were mild or moderate.

The proposed baseline anti-AAV5 neutralizing antibody threshold (1:900) of the indication was considered justified based on currently available data. However, the number of subjects and duration of follow-up were limited, and more clinical data is necessary. Besides, anti-AAV5 neutralizing antibody assay is not a routine laboratory test, and different assays may have different results. The assay recommended by the Applicant is not available in Taiwan, and the Applicant should clarify the ways to screen anti-AAV5 neutralizing antibody for patients in Taiwan.

There was one case of hepatocellular carcinoma. Although these events were considered unrelated to etranacogene dezaparvovec infusion, long-term follow-up is still necessary.

More data from Asian subjects are necessary and should be submitted once available.

Risk management plan is also necessary, including the long-term follow-up plan for patients in Taiwan.

In conclusion, CDE review team leader still recommends approval of etranacogene dezaparvovec.

1. The recommends regular approval of Hemgenix for the indication: "Hemgenix is indicated for the treatment of adults aged 18 and older with severe and moderately severe Hemophilia B requiring Factor IX prophylaxis therapy, without a history of Factor IX inhibitors, and with pre-existing neutralizing antibodies to adeno-associated virus serotype 5(AAV5) titer below 1:900."
2. The recommended dosage is 2×10^{13} genome copies (gc) per kilogram of body weight, administered as a single intravenous infusion.
3. The stability results support the shelf life of the drug product protected from light at 5 ± 3℃ for 18 months.
4. Pre-approval requirements: The manufacturer must design and develop a comprehensive plan for conducting post-approval AAV5 neutralizing antibody testing in the domestic market for the purpose of screening patients eligible to receive this therapeutic product.
5. Post-approval requirements:
   (1) Final clinical study report upon completion of CT-AMT-061-02 clinical trial.
   (2) Final Clinical Study Report upon completion of CSL222_3002 in Japan.
   (3) Final Clinical Study Report upon completion of CSL222_3005.
   (4) For the CSL222_3003 trial, the clinical trial report should be submitted according to the timeframe specified in the EMA Risk Management Plan (RMP).
   (5) For the post-marketing long-term observational study CSL222_4001, the clinical study report should be submitted in accordance with the timeframe specified in the EMA Risk Management Plan (RMP).
6. A domestic risk management program shall be implemented, which should include long-term follow-up of domestic patients receiving the treated product.

2. Regulatory background

2.1 Worldwide Status in Regulatory Agencies

Hemgenix is approved for the treatment of hemophilia B in several countries including US and EU. The approved indication by USFDA and EMA is presented in the table below.

	Indication
US (2022/11/22)	HEMGENIX is an adeno-associated virus vector-based gene therapy indicated for the treatment of **adults with Hemophilia B** (congenital Factor IX deficiency) who: **• Currently use Factor IX prophylaxis therapy, or** **• Have current or historical life-threatening hemorrhage, or** **• Have repeated, serious spontaneous bleeding episodes.**
EU (2023/2/20)	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.**

2.2 Regulatory history in Taiwan

CDE Case Number	Description and Result
112RRC11318	少數嚴重疾病藥品審查認定。符合。
112RRC11319	優先審查認定。符合。
113IND02040	CSL222_3005 試驗申請於我國執行，本試驗為一Phase 3b, open-label, multicenter 試驗，檢測CSL222(Hemgenix)用於 pre-existing anti-AAV5 Neutralizing antibodies 之B 型血友病患者的療效反應與出血風險；此試驗亦為 USFDA PMR 之一。**申請者於本案中來函說明預計於我國收納 10 名受試者**。Approved.

2.3 Consideration of Bridging Study

There were only 2 Asian subjects data submitted by the sponsor and it is unable to confirm whether the two Asians are East Asian or not. At this time, clinical data from East Asians is limited. Given that clinical bleeding is primarily dependent on the degree of FIX deficiency, it is expected that race-related differences in efficacy will be minimal for the same FIX level. Based on previous experience with a similar gene therapy product (e.g. AAV-5 based gene therapy, ROCTAVIAN 112NDA05034) and the pharmacodynamic nature of Hemgenix, ethnic differences are expected to be negligible. The applicant has indicated that there are currently two ongoing studies in East Asia (CSL222_3002, Japan, initiated September 2023). CSL222_3005 is a multinational study that includes Taiwan (113IND02040, protocol approved. 10 subjects planned in Taiwan) and was initiated in 2024/1. Overall, the bridging study could be Conditional waive with PMR of CSL222_3005 and CSL222_3002 study.

3. Chemistry, Manufacturing and Controls Evaluation

3.1 Introduction

Biologics category:	Gene therapy
General Summary:	
Drug substance:	Etranacogene dezaparvovec (recombinant non-replicating adeno-associated virus serotype 5 vector comprising a codon-optimized coding DNA sequence for human coagulation factor IX variant R338L (FIX-Padua))
Starting material/ Expression system:	[redacted] cell line
Product name & strength:	Hemgenix Etranacogene dezaparvovec 1 x 10^{13} gc/ml; 10 mL
Pharmaceutical form:	Concentrate for solution for infusion
Sponsor:	傑特貝林有限公司
Manufacturer	DS, DP and Primary Packaging: UniQure Inc. Secondary Packaging and QP release: [redacted]
Indications:	Etranacogene dezaparvovec 為基因療法，以腺相關病毒為載體，已核准用於患有 B 型血友病(先天缺乏第九凝血因子)，且既有AAV5 中和抗體效價低於 1:900 的成人病人，以降低出血事件頻率，及滿足對第九凝血因子補充療法的需求，病人應： 目前使用第九凝血因子預防性療法，或目前或過去曾發生有生命危險的出血，或重複、嚴重自發性出血事件。
Routes of administration:	IV Infusion
Doses (s):	Hemgenix is a single dose of 2 x 10^{13} gc/kg body weight corresponding to 2 mL/kg body weight, administered as an intravenous infusion after dilution with sodium chloride 9 mg/mL (0.9%) solution for injection
Worldwide regulatory status:	US FDA: 2022.11 EMA: 2023.02
Full report	Team report format: ☐ RBRS ☐ Abridged report ■ Full report The reasons if it is not applicable:

General introduction

Nomenclature

International non-proprietary name (INN):		etranacogene dezaparvovec
Chemical name	Abbreviated name:	AAV5-hFIXco-Padua
	Long name:	Recombinant adeno-associated viral vector containing a codon-optimized coding DNA sequence for human coagulation factor IX variant R338L (FIX-Padua)
Company code name:		AMT-061 / CSL222
Other names:		rAAV5-FIXco-Padua
United States Adopted Name (USAN)		etranacogene dezaparvovec
Food and Drug Administration (FDA) designated suffix		-drlb
Chemical Abstracts Service (CAS) registration number:		2156583-26-3
Anatomical Therapeutic Chemical (ATC) Code:		B02BD16

Structure

AAV5-hFIXco-Padua is a recombinant adeno-associated viral vector containing a codon-optimized coding DNA sequence (CDS) for human coagulation factor IX variant R338L (FIX-Padua). The vector is composed of a linear single-stranded DNA-based vector genome that is encapsidated in an adeno-associated virus (AAV)-derived protein capsid.

[redacted]

[redacted]

[redacted]

[redacted]

General Properties

[redacted]

Mechanism of Action

Hemophilia B is characterized by the absence of functional factor IX (FIX) protein in the circulation, and the resulting clotting deficiency. Etranacogene dezaparvovec consists of a human FIXco-Padua (hFIXco-Padua) expression cassette, which is packaged within a recombinant adeno-associated virus of serotype 5, and administered by intravenous infusion into patients. The hFIXco-Padua expression cassette contains a codon-optimized 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. As a result, etranacogene dezaparvovec establishes secretion of functional FIX protein and partially or completely ameliorates the deficiency of

circulating FIX procoagulant activity of patients suffering from hemophilia B, thus restoring hemostatic potential and limiting bleeding episodes.

Physico-Chemical Characteristics

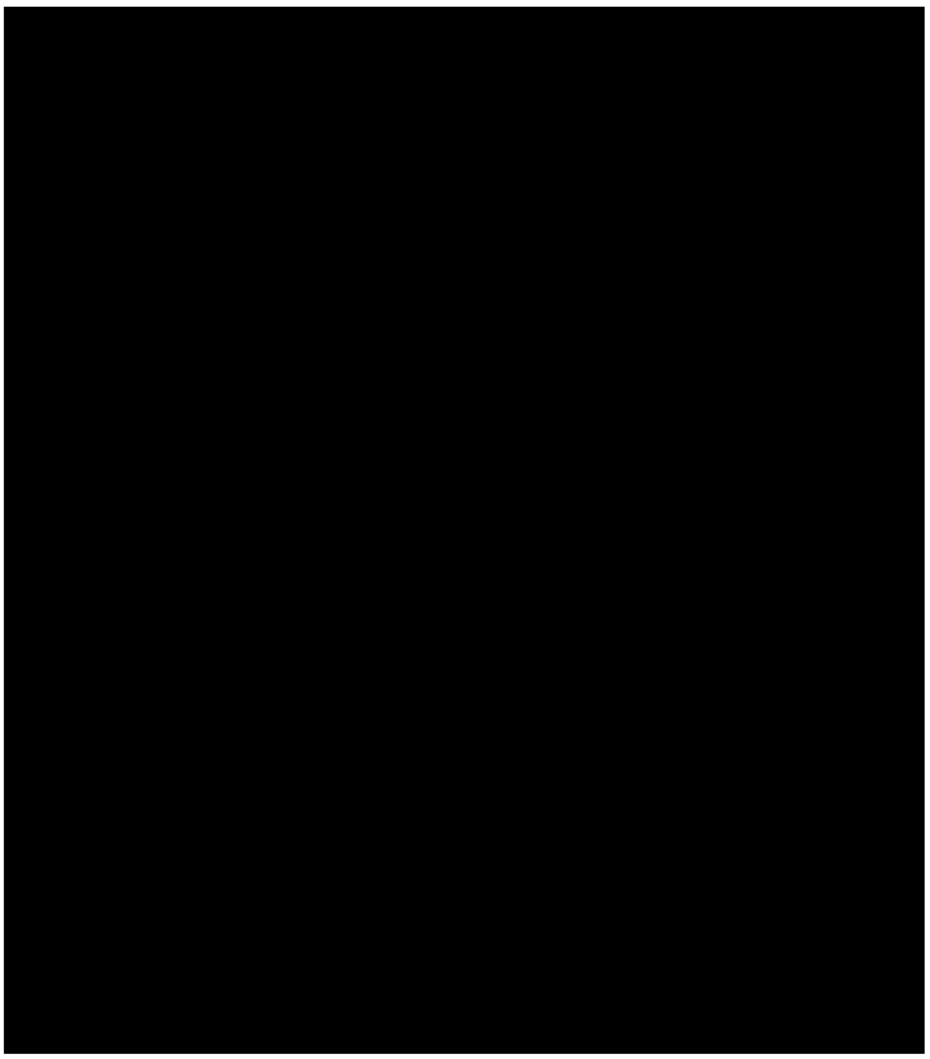
The Drug Substance is a clear, colourless solution that consists of etranacogene dezaparvovec in buffer composed of the final excipients of the Drug Product.

3.2 Drug Substance

3.2.1 Manufacturers

Name and Address of Manufacturer

DS Manufacturer: UniQure Inc. (113 Hartwell Avenue, Lexington, MA 02421-3125 USA)



[redacted]

3.2.2 Manufacturing Process and Process Controls

Batch Definition

One batch of DS is the product of the purification of material which is derived from a bulk product produced in [redacted] bioreactors. [redacted]

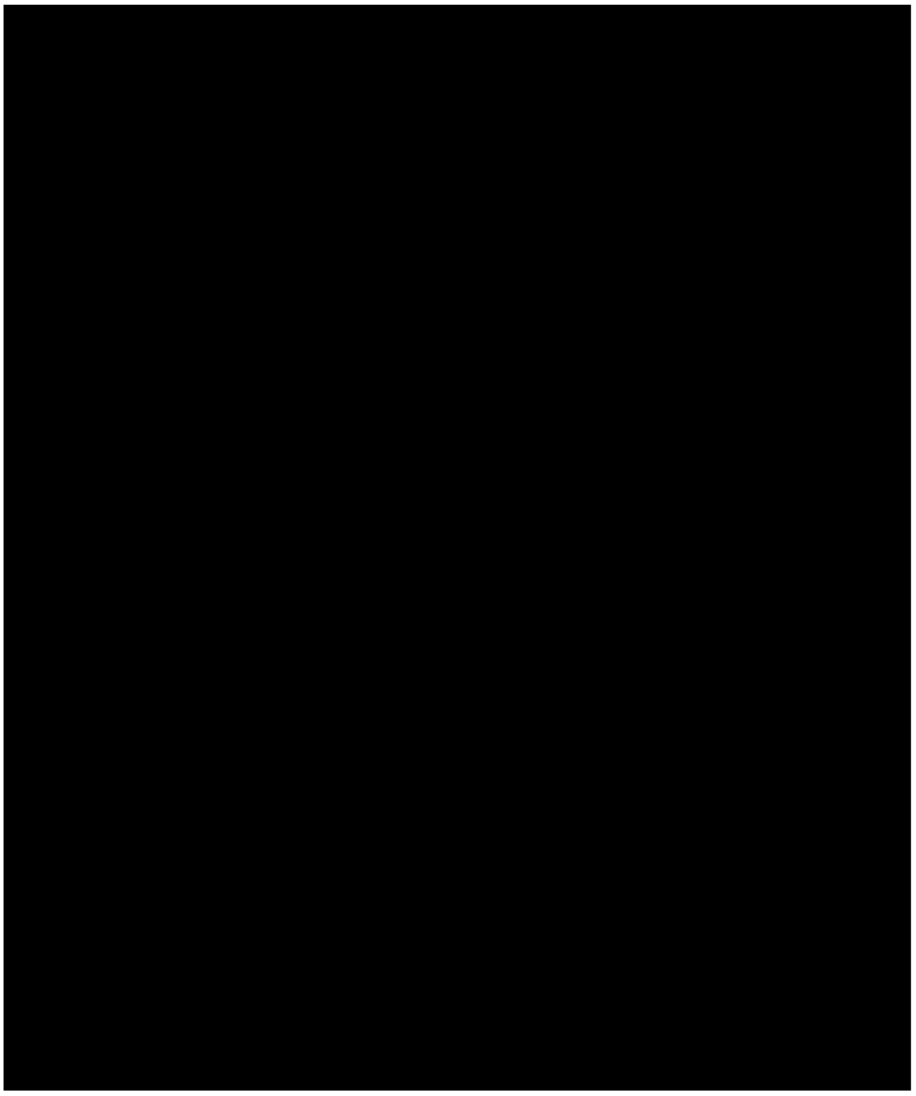
Baculovirus Expression Vector System

Etranacogene dezaparvovec DS is produced in separate production runs using the baculovirus expression vector system (BEVS) that utilizes a [redacted] insect cell line derived from *Spodoptera frugiperda* Sf9 cells [redacted] The BEVS is composed of [redacted] different recombinant baculoviruses, which serve to deliver the essential components to produce adeno-associated virus (AAV) containing the Padua variant of human factor IX gene (hFIXco-Padua) in the [redacted] cells. [redacted]

[redacted]

Master seed viruses (MSVs) and working seed viruses (WSVs) have been prepared for each of the [redacted] recombinant baculoviruses. Derivation and preparation of the viral banks is also provided.

[redacted]



Upstream Production of etranacogene dezaparvovec (to Crude Lysed Bulk)

[redacted]

[redacted]

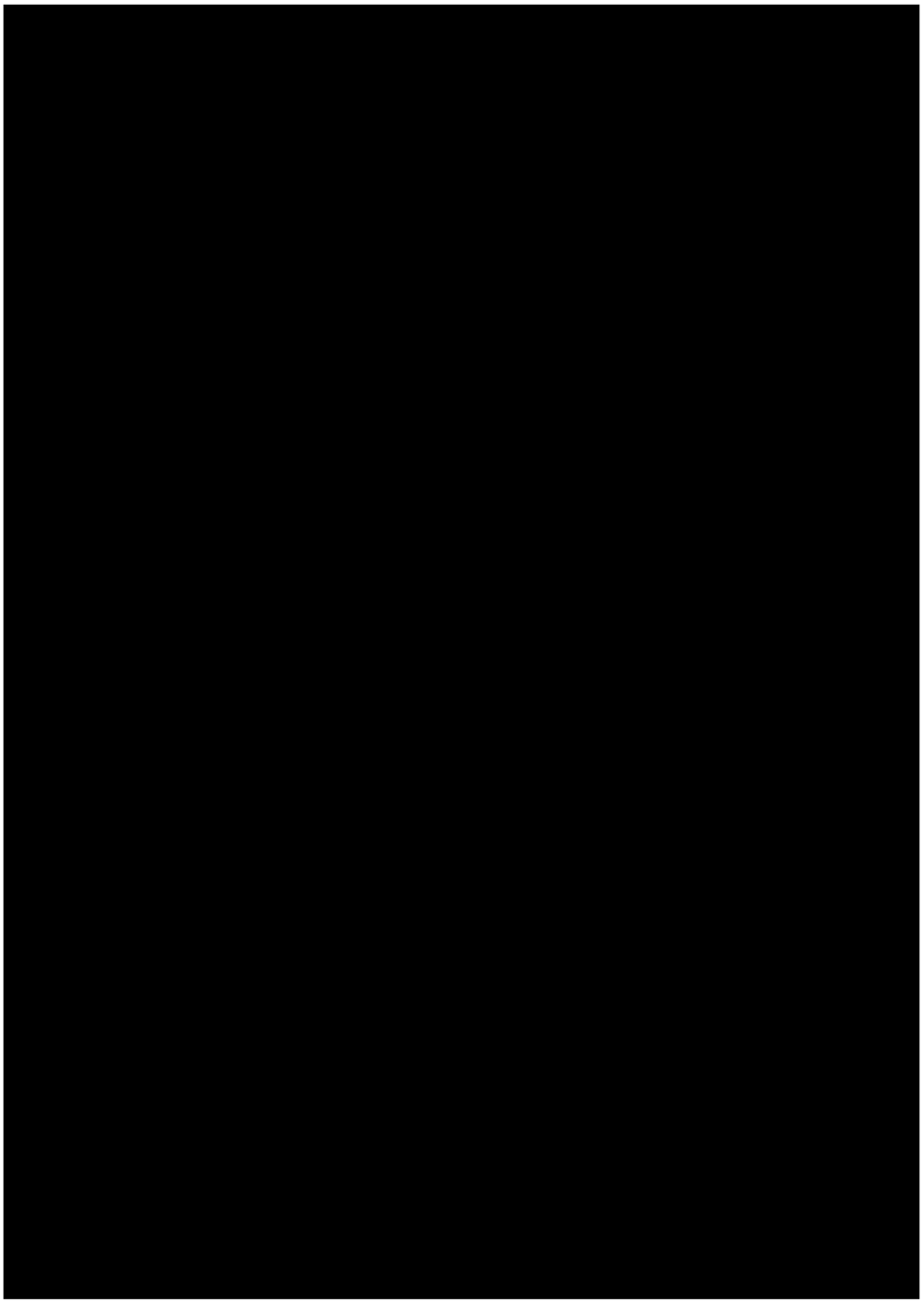
Downstream Purification (to Drug Substance)

[redacted]

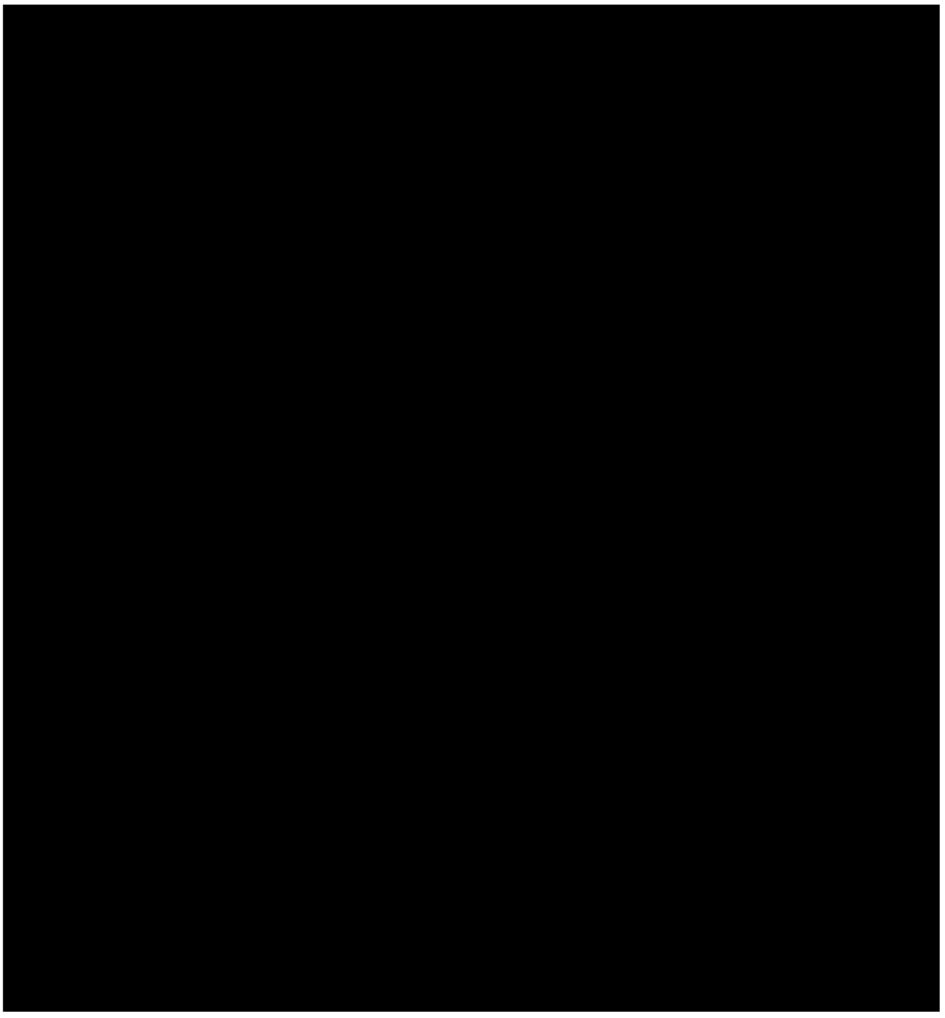
3.2.3 Control of Materials

- Preparation, Storage and Characteristion of Master/Working Cell Banks and Cells at the Limit of Age (CALs)

  History of the [redacted] Cell Line: Derivation and Culture [redacted]



- ✧ The MCB [redacted] for the [redacted] cell line [redacted] was characterized and tested [redacted] All test results met the requirements.
- ✧ Applied Testing for Characterization and Qualification [redacted] Cell Line



Genealogy of MCB, WCBs and CALs

Manufacturing details of MCB, WCBs and CALs

Tests Performed on the [redacted] MCB, WCBs and Their CALs

✧ The cells used for the manufacturing of etranacogene dezaparvovec are [redacted] an insect cell line. Specific consideration was given to the nature of the cell substrate, appreciating the susceptibility of the cell substrate to insect viruses that pose known risk to humans, [redacted] An assessment was made whether the selected virus tests can detect relevant arboviruses, [redacted] This assessment showed that [redacted]viruses can be detected by in vitro virus testing using indicator cell lines [redacted] and by in vivo virus testing using suckling mice in particular.

[redacted] the susceptibility of the cell substrate to other insect viruses was assessed. [redacted]

Retroviruses can be present in cell substrates including [redacted] cells. Therefore, testing of cell banks for retroviruses is included using a retrovirus co-culture test, [redacted].

[redacted]

[Reviewer's note]

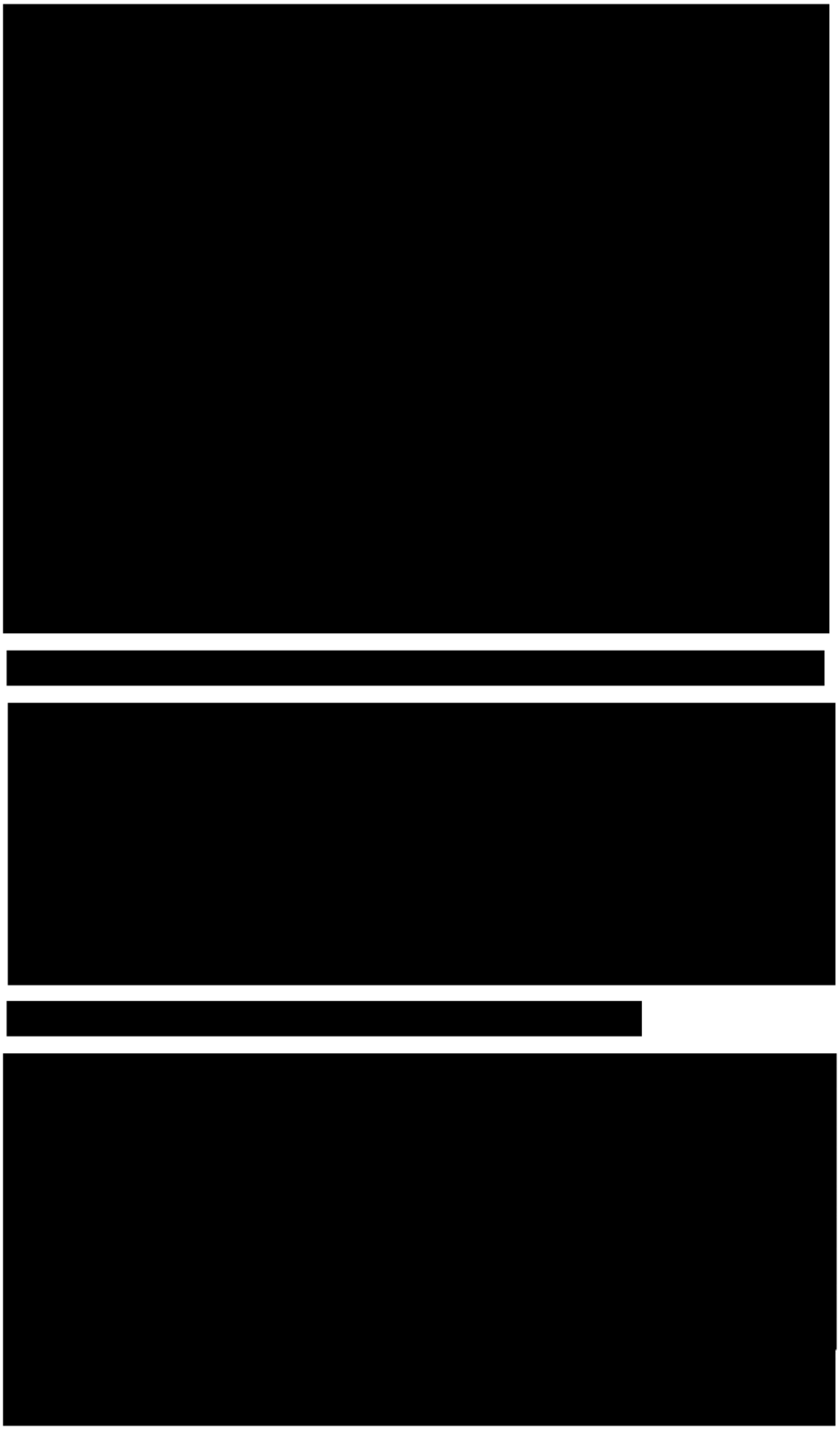
- All test results conformed to requirements. The identity of the MCB, WCBs and CALs was confirmed for all lots. No adventitious virus was detected in any lot tested. Purity testing met the requirements for MCB and WCBs. [redacted]

[redacted]

Cell Bank Stability

As part of cell bank qualification, vials from the master cell [redacted] and working cell banks ([redacted] are [redacted]. [redacted] MCB and WCBs are assessed for manufacturing suitability [redacted]. [redacted]

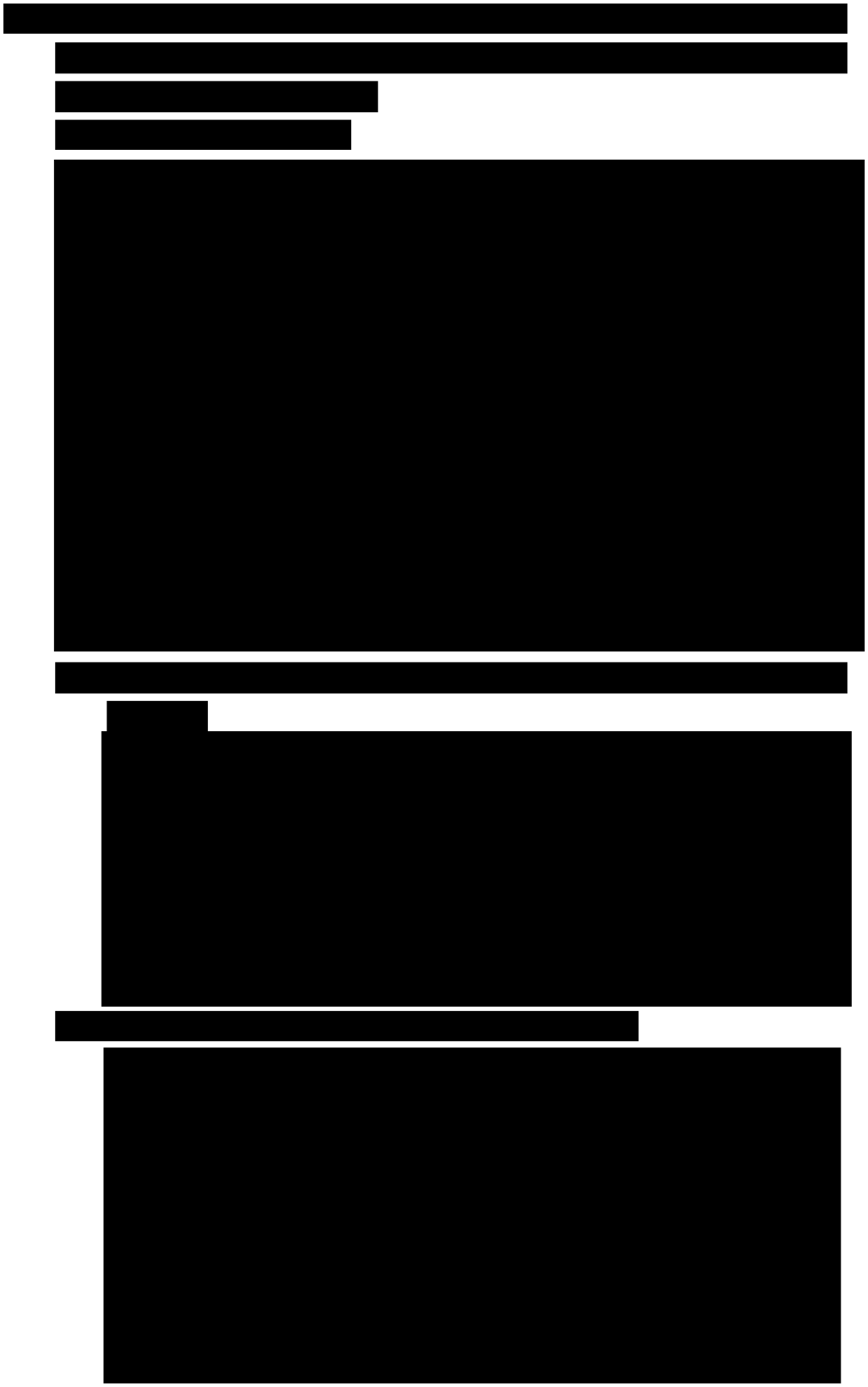
[redacted]



- Recombinant Baculoviruses

  Introduction

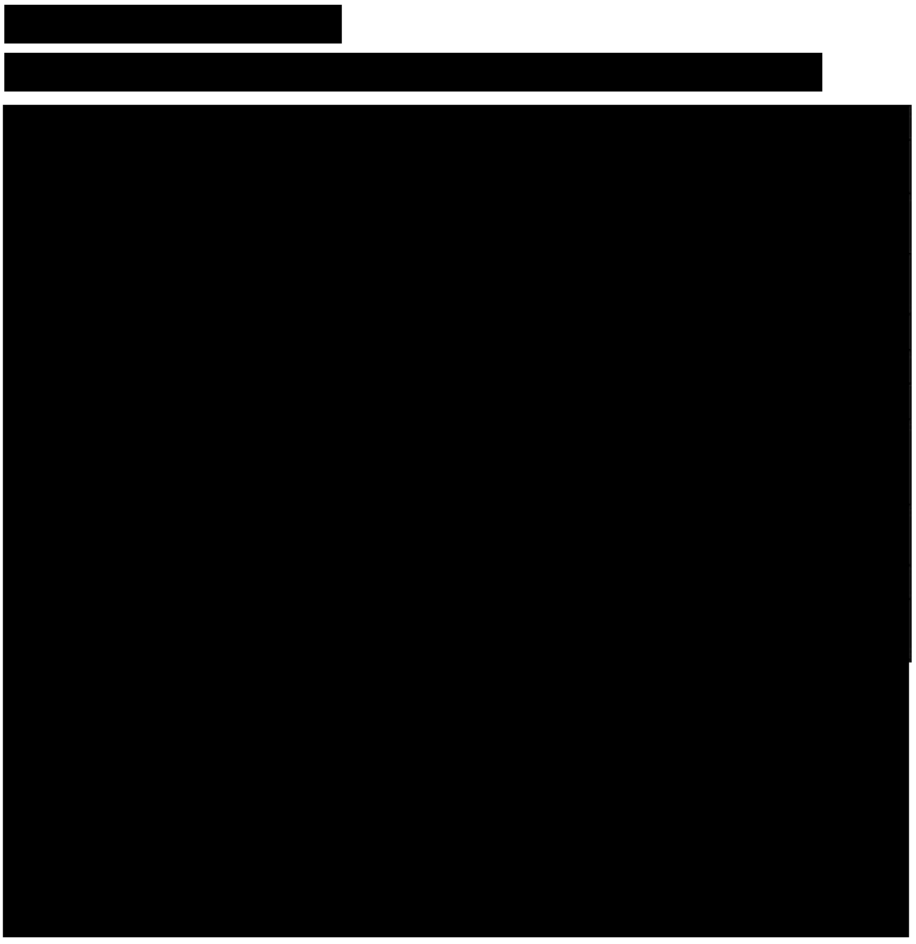
  Manufacture of etranacogene dezaparvovec involves co-infection of [redacted] cells with [redacted] recombinant baculoviruses. [redacted]



[redacted]

- Elements of the AAV5 Capsid Expression Cassette

[redacted]



The characterization studies of the selected recombinant baculoviruses [redacted] were considered acceptable for production [redacted]

[redacted]

[redacted]

MSVs and WSVs used in the manufacture of etranacogene dezaparvovec

[redacted]

[redacted]

[redacted]

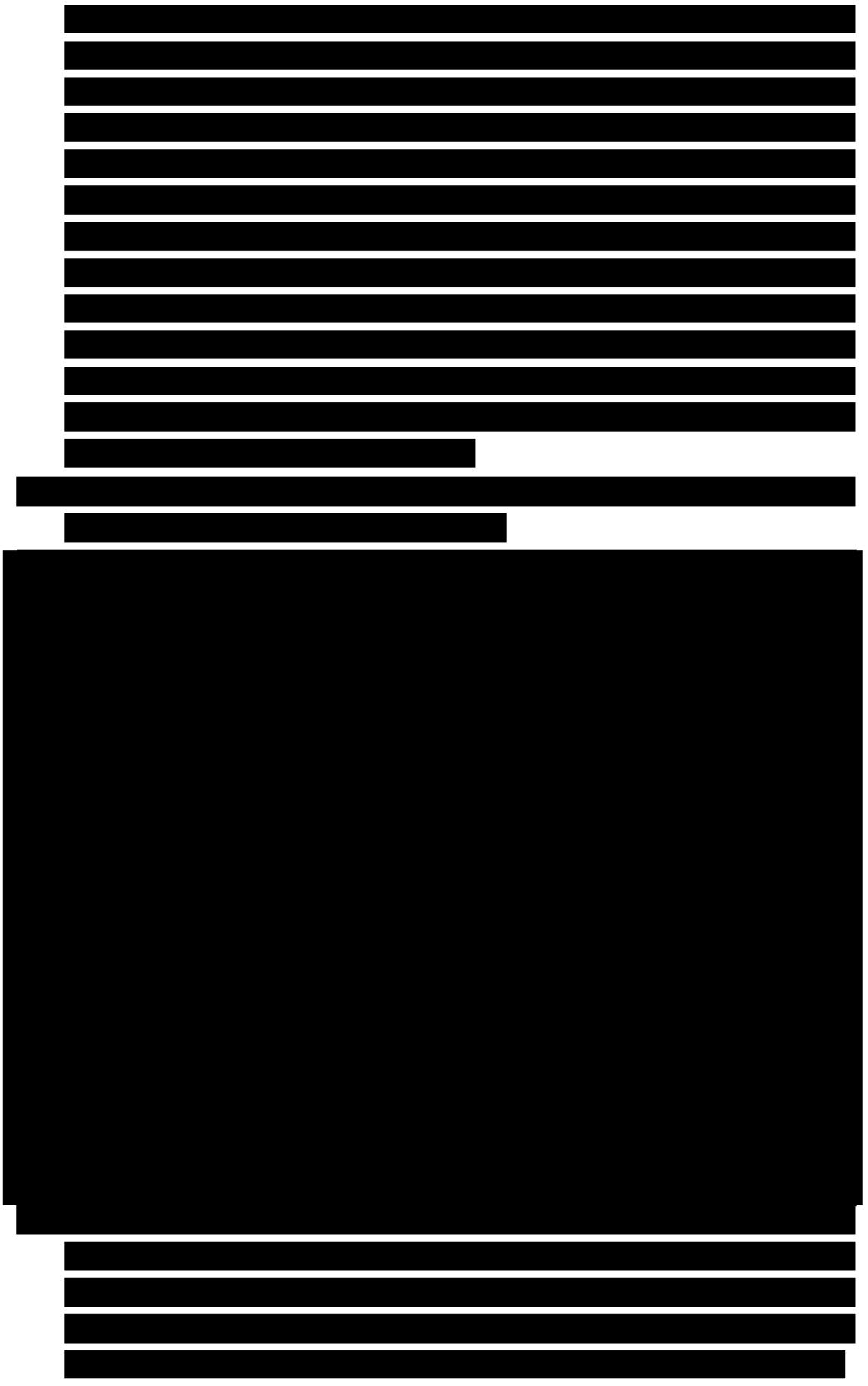
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]



[redacted]

GMP Manufacturing Facilities Used for MSVs and WSVs Production

The MSVs and WSVs have been produced in [redacted] Good Manufacturing Practices (GMP) facilities [redacted]

[redacted]

[redacted]

Stability of the MSVs and WSVs

Baculovirus infectivity for initial qualification and requalification of the master seed viruses (MSVs) and working seed viruses (WSVs) are executed to confirm the stability and maintain suitability of the viral seeds for use in the manufacturing process. [redacted]

[redacted]

- Other Raw Materials

[redacted] Raw Materials Used in the Manufacture of Etranacogene Dezaparvovec

[redacted]

[redacted] Raw Materials Used in the Manufacture of Etranacogene Dezaparvovec

[redacted]

✧ Cell Culture Media

[redacted] **The complete media composition** [redacted] **medium is proprietary,** [redacted]

[redacted]

- [redacted]
- **The filters are [redacted] from the vendor.**

[redacted]

Downstream Process Solutions

[redacted]

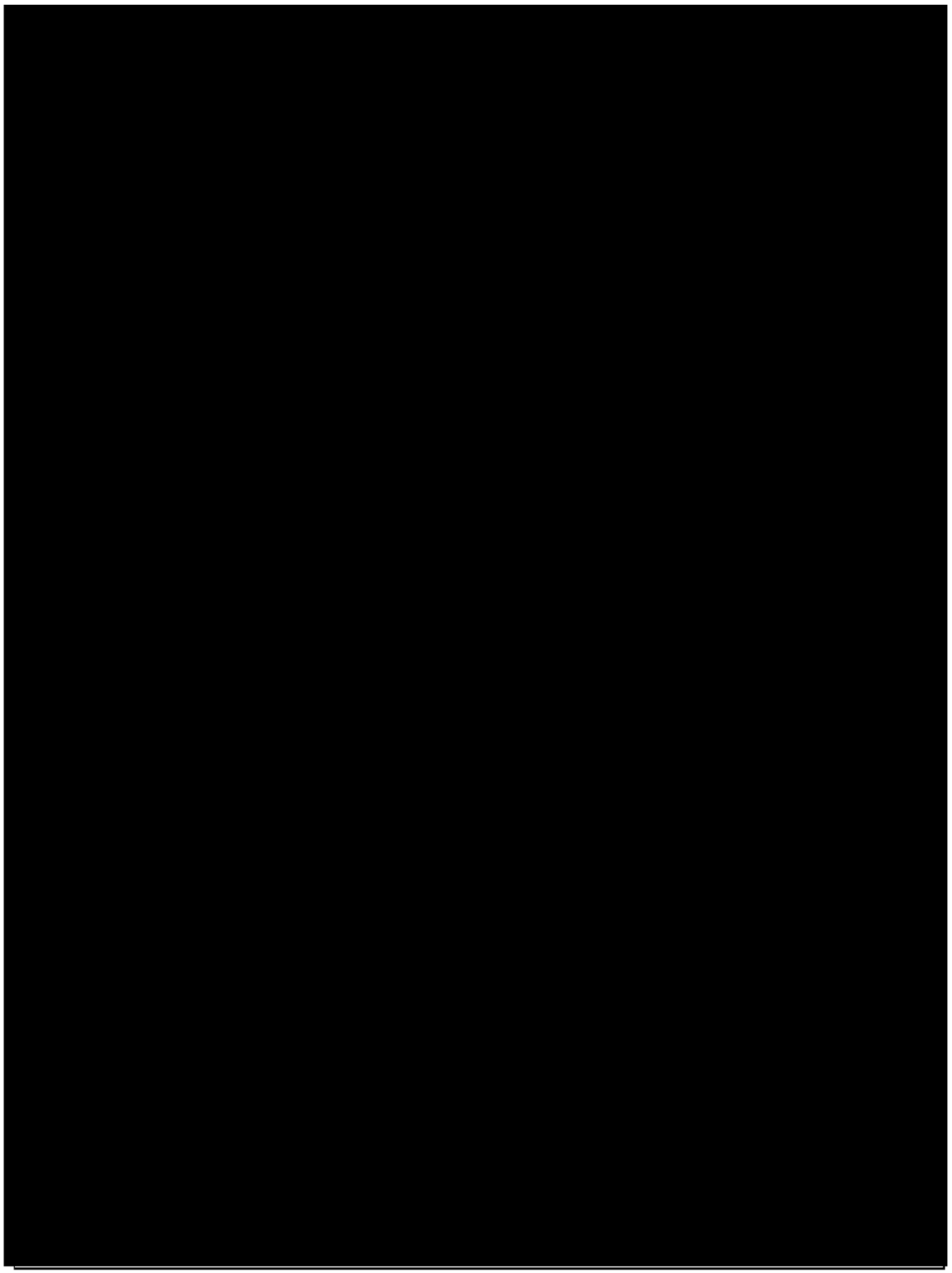
Raw Materials of Biological Origin Used in the Production of Etranacogene Dezaparvovec

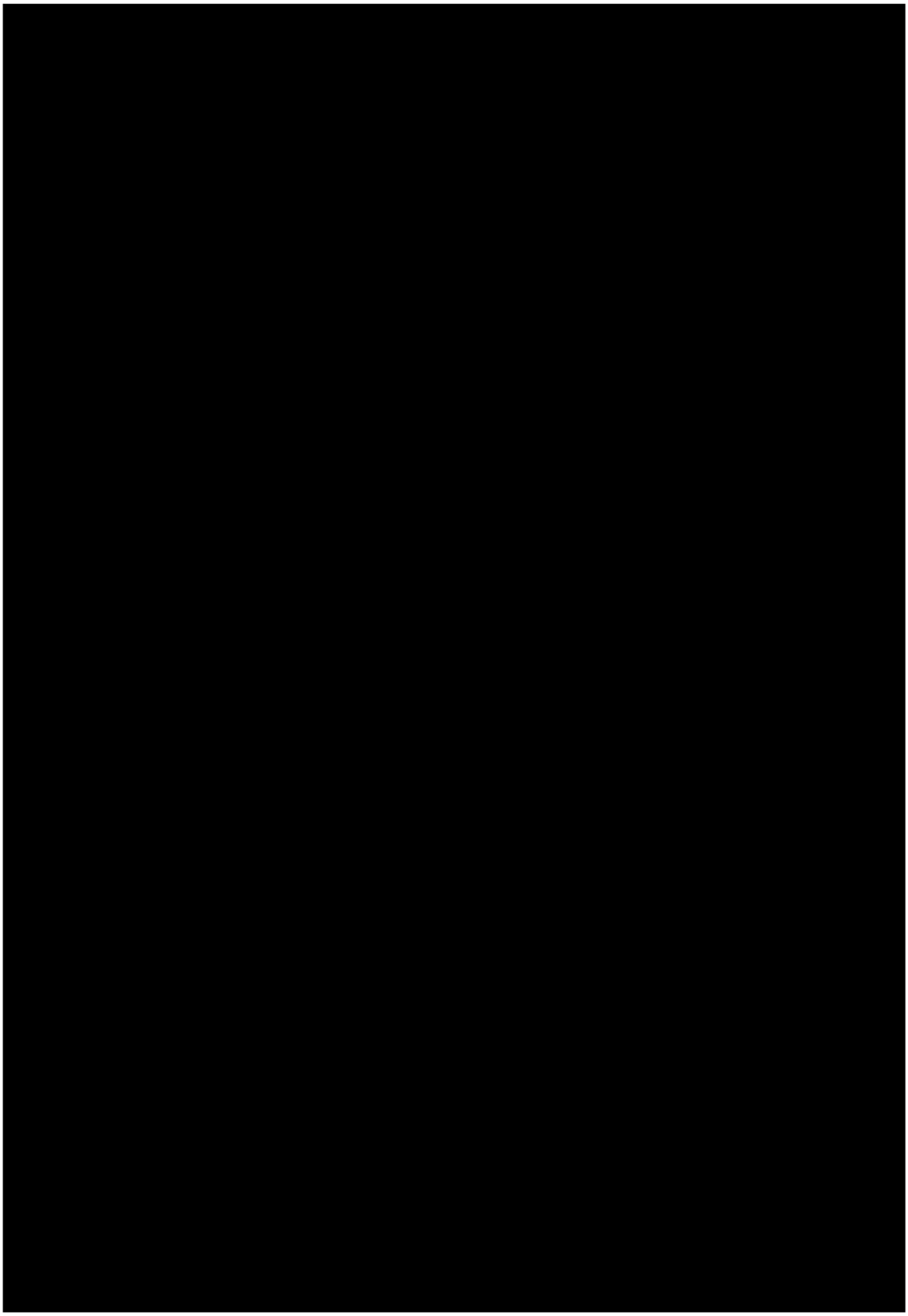
[redacted]

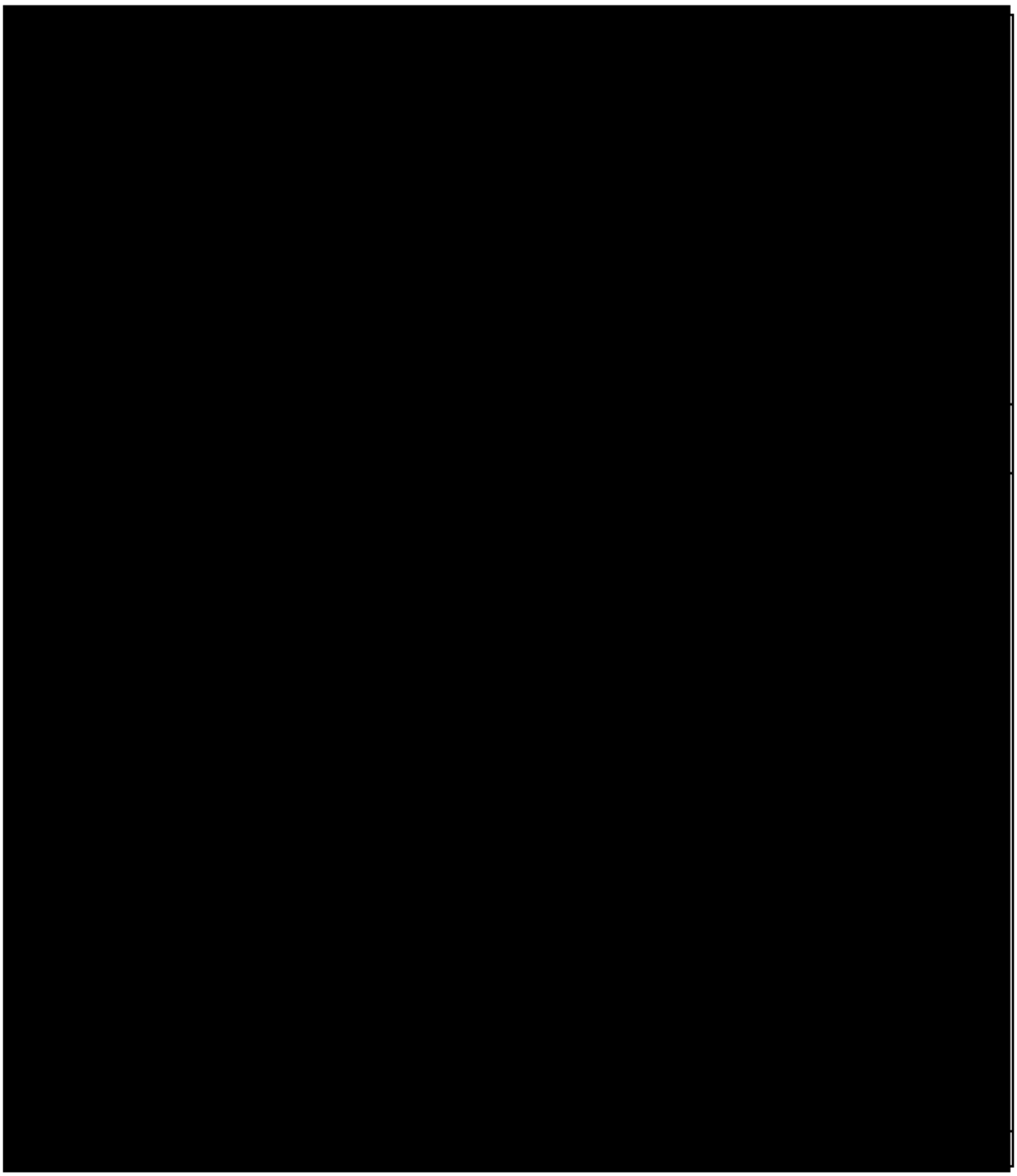
[Reviewer's note]

The extractables and leachables risk assessment [redacted] is provided.

3.2.4 Controls of Critical Steps and Intermediates







[redacted]

Control of Critical Intermediates

Process Intermediates Hold Time

[redacted]

[Reviewer's note]

[redacted]

Suggest analyzing the range of baculovirus MOI at each upstream process steps in historical batches and evaluate the addition of MOI control measures. Alternatively, please provide a rationale for why additional measures may not be necessary.

[redacted]

Following the [redacted] step, each baculovirus is blended [redacted] added to each bioreactor. [redacted]

[redacted] the etranacogene dezaparvovec process has several surrogate controls in place as described above to achieve consistent product quality.

Control of the baculovirus titer [redacted] is assured [redacted] An upper limit for [redacted] is not deemed necessary [redacted].

3.2.5 Process Validation and Evaluation

The process validation strategy employs a 3-stage risk-based approach to the process validation lifecycle:

Stage 1, Process Design

Stage 2, Process Qualification

Stage 3, Continued Process Verification

At each phase of the validation lifecycle, the product CQAs and process performance indicators are used to define the scope of work and burden of proof necessary to demonstrate the manufacturing process reliably and consistently produces product of appropriate quality. The process validation / process performance qualification (PV/PPQ) batches were performed to demonstrate that the process, when operated within the defined ranges, produces DS that consistently meets all IPCs, IPSs, and release specifications.

- Process Qualification
  - ✧ Facility Design, Qualification of Utilities and Equipment

    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 DS. All equipment, utilities, and facilities were qualified prior to use in the PV/PPQ.
  - ✧ Process Validation / Process Performance Qualification

    The PV/PPQ confirms that the process design and Process Control Strategy (PCS) for the etranacogene dezaparvovec DS commercial manufacturing process perform as expected and consistently produce product of the specified/defined quality. [redacted]

[redacted]

A risk-based approach was used to determine the number of batches required for PV/PPQ. The risk assessment concluded that the number of clinical, Engineering, and at-scale development batches [redacted] provided sufficient process knowledge and provided satisfactory information regarding process variation. For etranacogene dezaparvovec DS, [redacted] consecutive DS batches, meeting all PV/PPQ acceptance criteria were considered sufficient to validate the manufacturing process.

A summary of the results for the PV/PPQ batches are provided below.

✧ Process Validation / Process Performance Qualification Results

The etranacogene dezaparvovec process performance qualification/process validation protocol was executed at uniQure's manufacturing facility located in Lexington, MA, USA. The PV/PPQ protocol [redacted] specifies a successful PV/PPQ campaign as containing [redacted] consecutive DS batches satisfying predefined acceptance criteria. A PV/PPQ campaign consisting [redacted] batches was executed in accordance with [redacted], and all batches were performed per current effective batch records. All [redacted] batches were evaluated against predefined acceptance criteria as outlined with the PPQ/PV protocol.

[redacted]

[Reviewer's note]

The PV/PPQ results from this etranacogene dezaparvovec DS manufacturing campaign met acceptance criteria to qualify the commercial manufacturing process. The PV/PPQ runs demonstrated the capability of the process and ensured the DS manufacturing process is reliable, repeatable, and consistently operating in a state of control.

- ✧ Process Intermediate Hold Times

  [redacted] data to support process intermediate hold times is provided. The hold times for the process intermediates [redacted] are limited to the maximum hold times confirmed [redacted] in the full-scale hold time validation study also provided and the times are reflected in Section 3.2.4.

- Continued Process Verification

  A continued process verification (CPV) program for etranacogene dezaparvovec DS is part of the overall Process Validation program and is currently ongoing. Throughout the lifecycle of the product, a statistical evaluation of the data will be performed to demonstrate that the process remains in a state of control. The CPV program will support the Annual Product Review program.

  [redacted]

3.2.6 Manufacturing Process Development

Introduction

The goal of the manufacturing process development was to establish a robust commercial manufacturing process capable of consistently producing etranacogene dezaparvovec of the intended product quality. [redacted]

[redacted]

The cumulative process understanding that was gained [redacted] was used to establish the control elements, process parameters, and material attributes, which will be implemented during the commercial manufacturing process.

[redacted]

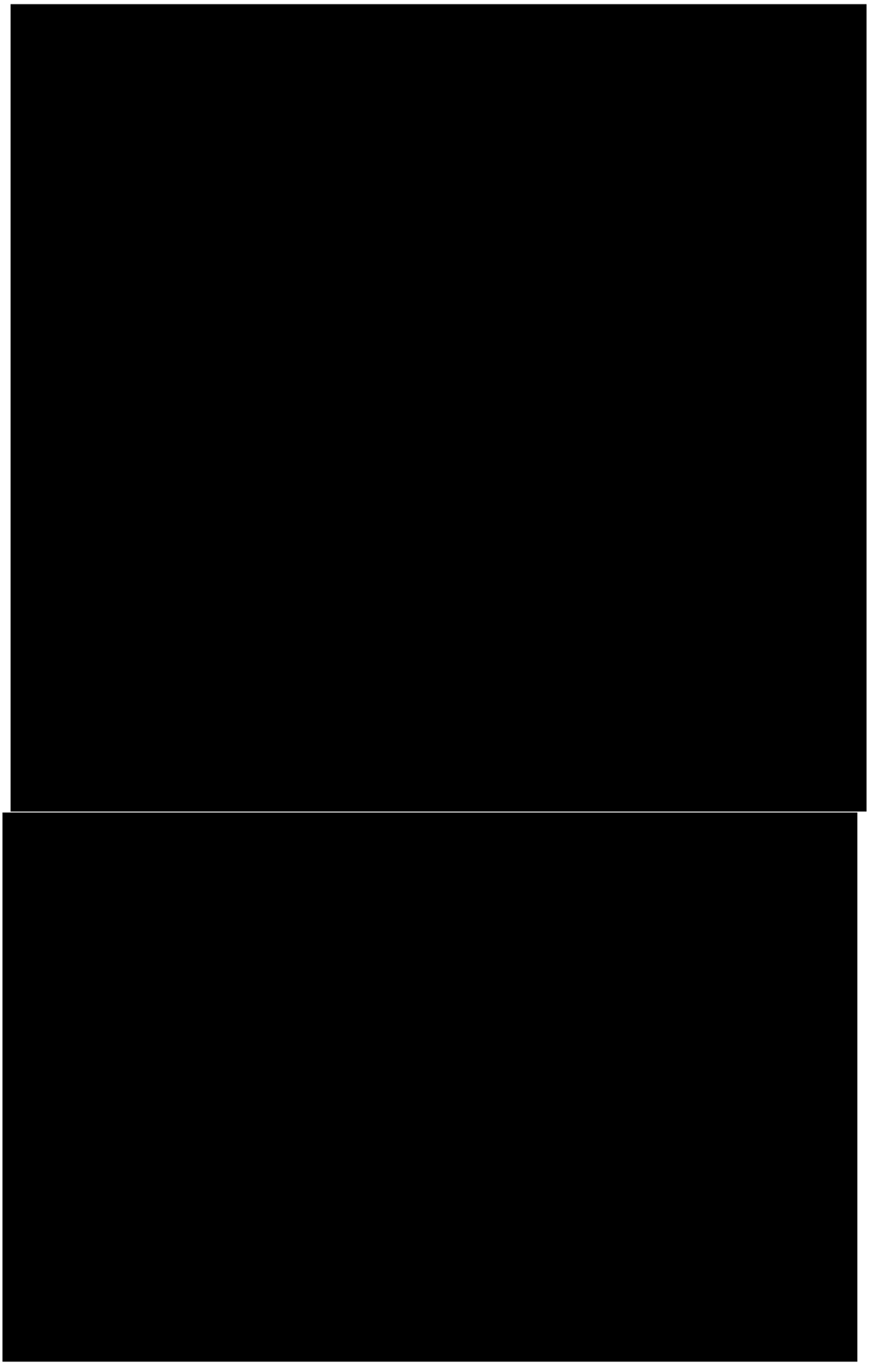
[Reviewer's note]

Comparability assessment [redacted] are described. The comparability assessment included [redacted] studies using material [redacted]. The non-clinical comparability and the clinical comparability are also described. At each stage of development, the comparability assessments supported proceeding to the next stage.

3.3 Characterization

3.3.1 Characterization of drug substance

Summary of Etranacogene Dezaparvovec DS, DP and RS Batches Used in Characterization Studies



3.3.2 Impurities

Summary of Product and Process-related Impurities as Assessed [redacted] in Etranacogene Dezaparvovec DS Batches used in Non-clinical and Clinical Studies

[redacted]

Summary of Process and Product-related Impurities in Etranacogene Dezaparvovec DS Batches Used for PV/PPQ

Impurity	Release Test Method[c]	Result Unit	PV/PPQ Batches[a, b] (N=6) [redacted]	Range of all PV/PPQ Batches	Range of Non-clinical and Clinical Batches (Table 3)
Product-related Impurities					
[redacted]					
Aggregates[f]					
Replication-competent AAV					
Process-related Impurities-derived from upstream process					
Residual Host Cell Proteins					
[redacted]					
Residual DNA					
Residual Baculovirus DNA[g]					
[redacted]					
Residual Infectious Baculovirus					
Process-related Impurities-derived from downstream process					
[redacted]					

Product-related impurities

[redacted]

Process-related impurities

[redacted]

[Reviewer’s note]

[redacted]

[redacted]

[redacted]

3.4 Control of drug substance

Specification for Drug Substance ([redacted])

Attribute Monitored	Method	Acceptance Criteria
General Tests		
Appearance[a] - Color - Clarity	[redacted]	
Polysorbate-20 Concentration		
Identity		
Vector DNA Identity		
Content		
Biological activity		
Potency[a]		

[Reviewer's note]

[redacted]

Validation of Analytical Procedures

Analytical methods have been validated in accordance with ICH guidelines or verified according to Ph. Eur./USP (compendial methods) as applicable.

Batch Analysis

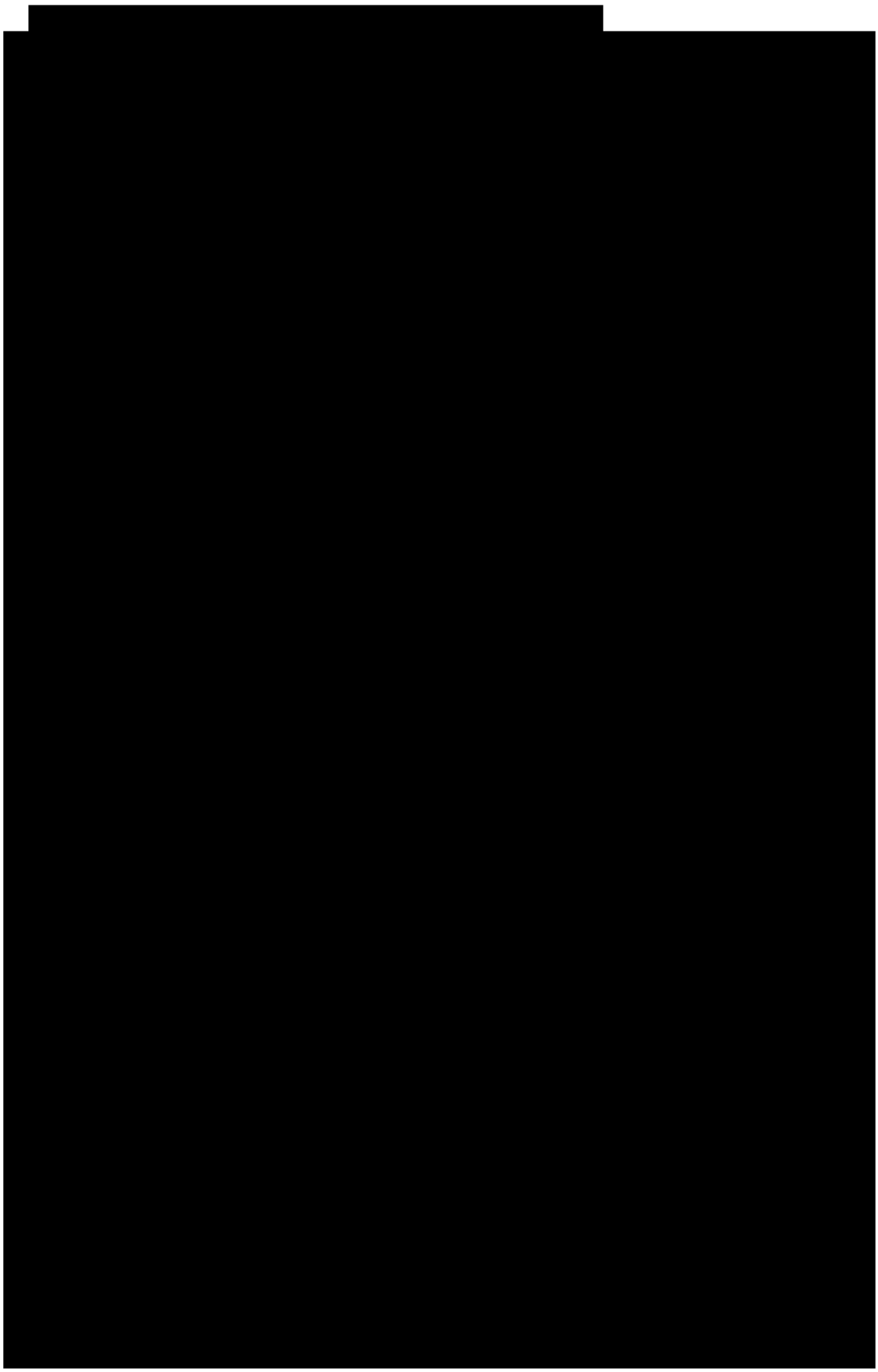
[redacted]

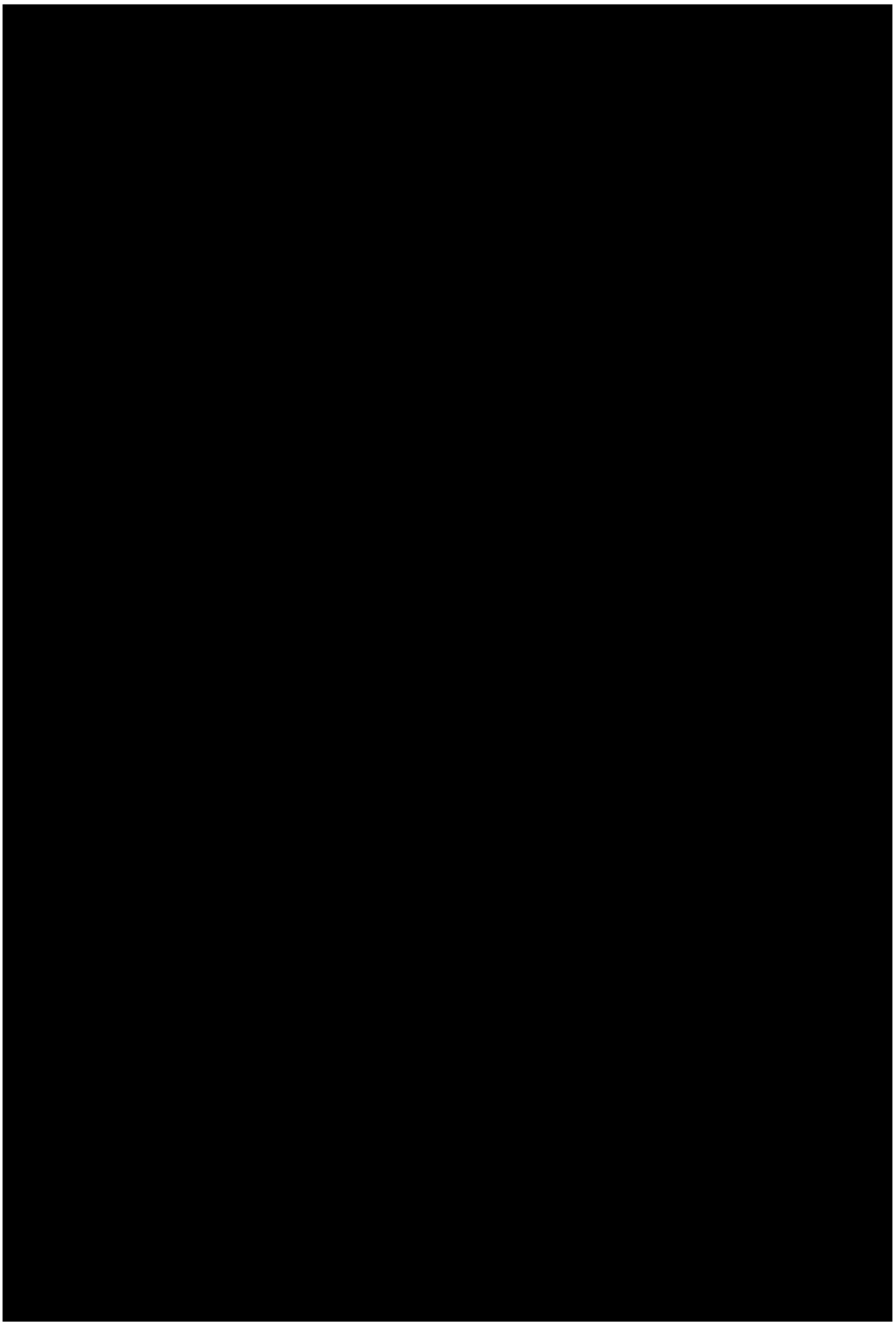
Justification of Specification

Overview

Justifications of the release and shelf-life specifications for drug substance (DS) are provided. The current DS specifications were established based on a statistical analysis of historical manufacturing data including assay capability, risks to process and/ or patients associated with variations within these stated product attributes as well as non-clinical and clinical experience.

[redacted]





[redacted]

3.5 Reference standard

The product derived primary reference standard (PRS) used for drug substance (DS) batch release and stability testing is the same as for drug product testing [redacted] is described [redacted].

3.6 Container closure system

✧ No novel components are utilized as a part of the container closure system.

General Information

[redacted]

Drug Substance Container Closure System

[redacted]

The biocontainer complies with the following compendial standards:

[redacted]

[redacted]

Suitability for the Intended Application

The biocontainers are designed for storage, transfer and transport of biopharmaceutical fluids under sterile conditions. [redacted]

[redacted]

The extractables and leachables (E&L) risk assessment and integrity testing of the container closure system are also provided.

3.7 Stability

Stability Conclusions

Based on the real-time stability data from [redacted] batches [redacted] **shelf-life for DS** [redacted]

[Reviewer's note]

According the real-time long-term stability data, the shelf-life for DS stored -20°C ± 5°C is [redacted]

[redacted]

Container Closure System

The DS stability samples are stored in [redacted] the final container closure system [redacted]

Stability Data

- Long-Term Stability Studies

[redacted]

[redacted]

[redacted]

[Reviewer's note]

[redacted]

[redacted]

- Accelerated Stability

[redacted]

[redacted]

[redacted]

[redacted]

- Stressed Stability

[redacted]

[redacted]

[redacted]

3.3 Drug product

3.3.1 Composition and packaging material of the drug product

Product Description

The drug product (DP) is etranacogene dezaparvovec, concentrate for solution for infusion and is also known as etranacogene dezaparvovec, injection, for intravenous infusion. The proposed proprietary name for the DP is HEMGENIX. The DP is intended for administration after dilution as a single-dose intravenous infusion.

Etranacogene dezaparvovec is a preservative-free, liquid formulation with a nominal concentration of 1×10^{13} genome copies (gc)/mL in a single-use 10 mL glass vial and is formulated in a sterile phosphate buffered saline (PBS) solution, pH 7.1 containing sucrose [redacted] and polysorbate-20 (PS-20) [redacted] For pH adjustment of the buffer, small amounts of hydrochloric acid compliant to compendial standards are used.

Composition

[redacted]

Container

The DP is presented in [redacted] 10 mL Type I glass vial stoppered with [redacted] rubber stopper, and sealed with an aluminum flip-off cap.

3.3.2 Pharmaceutical Development

- Drug Product Formulation

[redacted]

- Manufacturing Process Development

[redacted]

[redacted]

[Reviewer's note]

[redacted]

[redacted]

- Container Closure System
  Please refer to Section 3.3.7.

- Microbiological Attributes
  Etranacogene dezaparvovec, concentrate solution for infusion, in 10 mL vials complies with compendial requirements for microbiological attributes as laid down in Ph. Eur. 5.20 Parenteral Preparations and USP <1> Injections monographs. This is a single dose drug product (DP) intended for administration as single dose intravenous (IV) infusion, and as such, no antimicrobial preservative is included in the preparation.
  Sterility of etranacogene dezaparvovec DP is assured during manufacture [redacted] Sterility of the DP is assured [redacted] and also monitored [redacted] to assure sterility [redacted].
  Protection from microbial contamination is achieved [redacted]

[redacted]

✧ Container Closure Integrity Testing (CCIT)

[redacted]

- Compatibility

  Etranacogene dezaparvovec will be administered as a single dose via IV infusion. Prior to administration to the patient, etranacogene dezaparvovec is diluted with saline solution (Ph. Eur./USP grade). A single dose contains 2×10^{13} gc/kg diluted in 0.9% sodium chloride. The amount and required volume of DP needed to prepare the dose is determined by the patient weight. The appropriate number of vials will be pooled to obtain the correct patient dose.

[redacted]

[redacted]

Compatibility has been demonstrated for the DP with [redacted] IV infusion sets used for administration into patients. [redacted]

Etranacogene dezaparvovec was also shown to be compatible with the procedures used to administer the dose to patients, including transportation of the diluted DP from the clinical pharmacy to the patient infusion center. **These data confirm the in-use stability of etranacogene dezaparvovec after dilution [redacted] and demonstrate the compatibility of with the IV administration dosage devices.** [redacted]

[Reviewer's note]

[redacted]

3.3.3 Manufacture

Manufacturer(s)

[redacted]

DP Manufacturer: UniQure Inc. (113 Hartwell Avenue, Lexington, MA 02421-3125 USA)

Batch Formula

- Batch Size

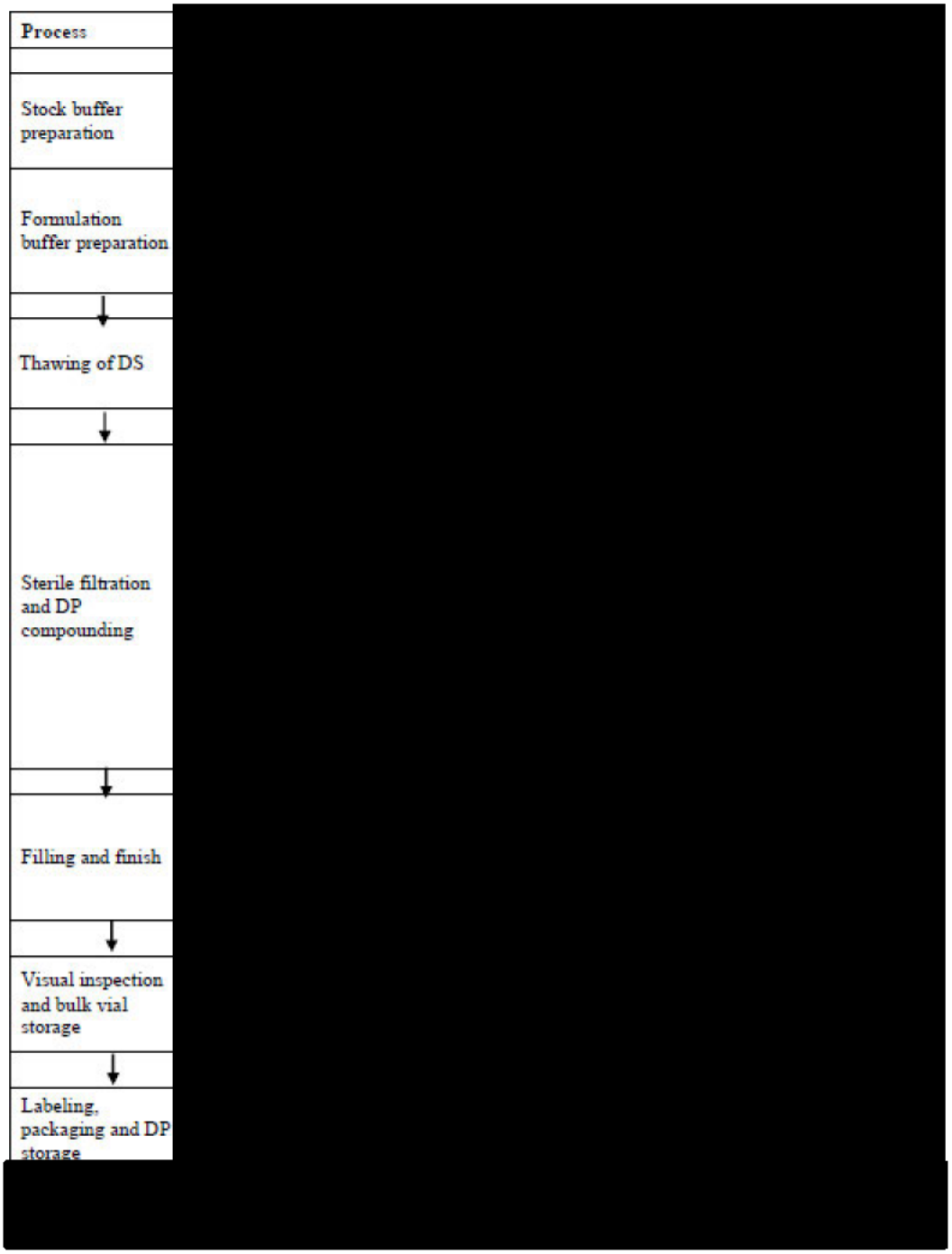
  Exemplary Etranacogene Dezaparvovec DP Batch Size

  [redacted]

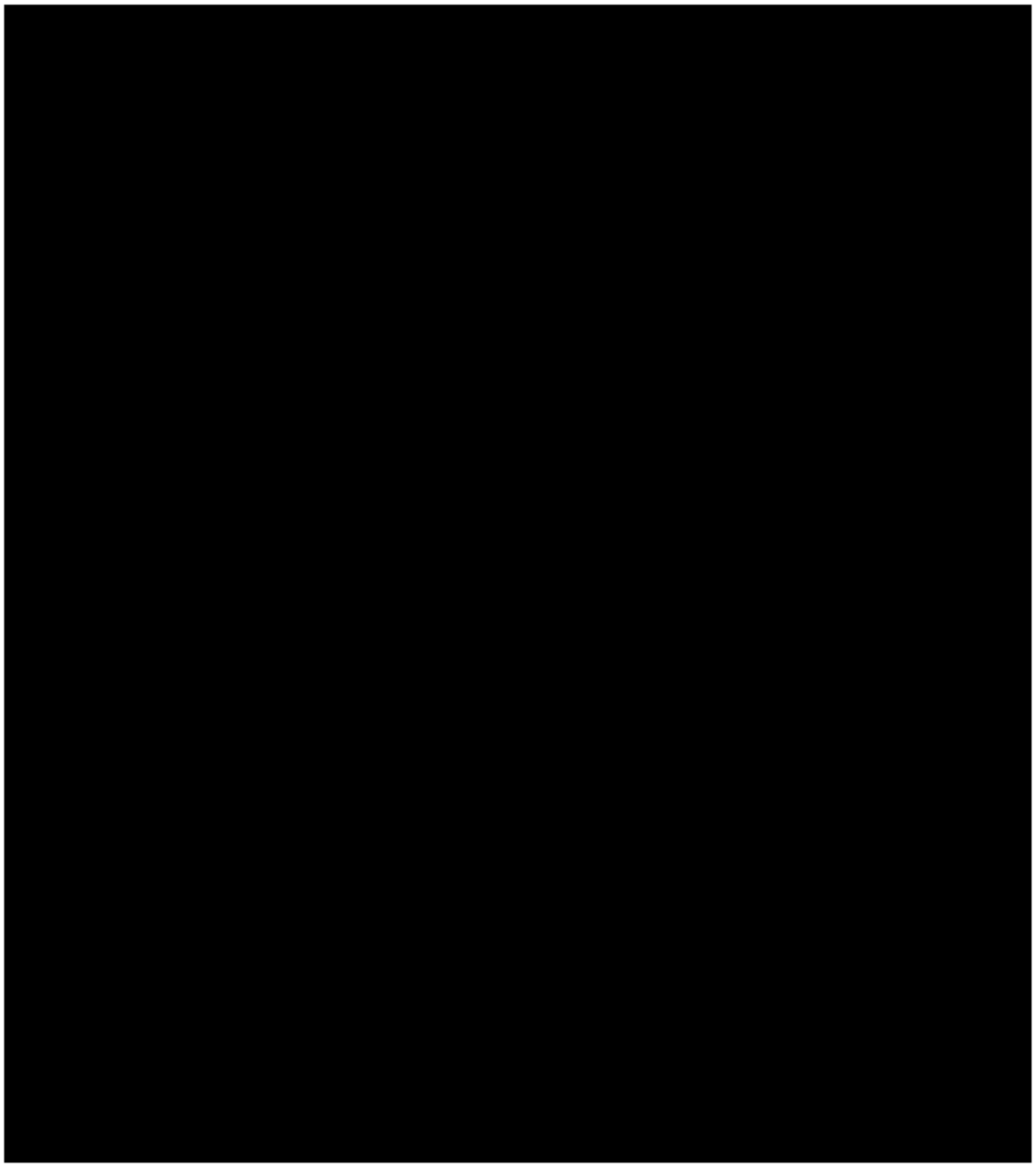
- Batch Formula

  [redacted]

Description of Manufacturing Process and Process Controls



Control of Critical Steps and Intermediates



Process Validation and Evaluation

The process validation strategy employs a 3-stage risk-based approach to the process validation lifecycle:

Stage 1, Process Design

Stage 2, Process Qualification

Stage 3, Continued Process Verification

At each phase of the validation lifecycle, the product CQAs and process performance indicators are used to define the scope of work and burden of proof necessary to demonstrate the manufacturing process reliably and consistently produces product of appropriate quality. The process validation / process performance qualification (PV/PPQ) batches were performed to demonstrate that the process, when operated

within the defined ranges, produces DP that consistently meets all IPCs, IPSs, and release specifications.

- Process Qualification
  - ✧ Facility Design, Qualification of Utilities and Equipment

    The PV/PPQ was performed at commercial scale [redacted] which is a qualified facility for the manufacture of etranacogene dezaparvovec. All equipment, utilities, and facilities were qualified prior to use in the PV/PPQ.
  - ✧ Process Validation / Process Performance Qualification

    The PV/PPQ confirms that the process design and process control strategy for the etranacogene dezaparvovec DP manufacturing process perform as expected and produce product of appropriate quality. The etranacogene dezaparvovec DP manufacturing process that includes Sterile Filtration and Drug Product Compounding, Drug Product Filling and Finish, Visual Inspection and Storage, and Buffer Preparation was qualified. [redacted]

    Hold times of the Thawed DS, Bulk DP [redacted] and Finished DP [redacted] were also included as part of the PV/PPQ [redacted]

    A risk-based approach is used to determine the number of batches required for PV/PPQ. The risk assessment includes the level of process and product knowledge [redacted], amount of clinical and engineering batch manufacturing experience [redacted] and technical complexity [redacted]. For etranacogene dezaparvovec DP, [redacted] consecutive DP batches, meeting all PV/PPQ acceptance criteria were considered required to validate the manufacturing process.

    A summary of the results for the PV/PPQ batches are provided below.
  - ✧ Process Validation / Process Performance Qualification Results

    [redacted] consecutive, successful etranacogene dezaparvovec DP PV/PPQ batches were manufactured [redacted]. The PV/PPQ batches manufactured are summarized in table below.

    Etranacogene Dezparovec Drug Product Process Performance Qualification Batches

[Reviewer's note]

The etranacogene dezaparvovec manufacturing process includes CPP and IPC range acceptance criteria

✧ Process Intermediate Hold Times

PV/PPQ Thawed DS and Bulk DP Hold Time Study Design

[Reviewer's note]

The PV/PPQ results from this etranacogene dezaparvovec DP manufacturing campaign met acceptance criteria to qualify the process with [redacted] consecutive and successful DP batches. The qualification runs demonstrated the capability of the process and ensured the Drug Product manufacturing process is reliable, repeatable, and consistently operating in a state of control.

- Continued Process Verification

  A Continued Process Verification program for etranacogene dezaparvovec DP is part of the overall Process Validation program and is currently ongoing. Throughout the lifecycle of the product, a statistical evaluation of the data will be performed to demonstrate that the process remains in a state of control.

  [redacted]

3.3.4 Control of excipients

Excipients in the Drug Product

Excipient	Quality Standard[1]
Hydrochloric acid	USP-NF, Ph. Eur., JP
Polysorbate-20	USP-NF, Ph. Eur., BP, JP
Potassium chloride	USP-NF, Ph. Eur., BP, JP
Potassium phosphate, monobasic	USP-NF, Ph. Eur., BP
Sodium chloride	USP-NF, Ph. Eur., BP, JP
Sodium phosphate, dibasic, anhydrous	USP-NF, Ph. Eur., JP
Sucrose	USP-NF, Ph. Eur., BP, JP
Water for injections	USP-NF, Ph. Eur.

[1] According to current version of the respective pharmacopoeia.

[Reviewer's note]

No excipients of human or animal origin are used in the DP.

No novel excipients are used in the manufacture of the DP.

3.3.5 Control of drug product

Specification for Drug Product [redacted]

Attribute Monitored	Method	Acceptance Criteria
General Tests	[redacted]	[redacted]
Appearance[a] - Color - Clarity - Visible Particulates		
[redacted]		
Sucrose Concentration		
Polysorbate-20 Concentration		
Subvisible Particles[a]		
Extractable Volume		
Safety		
Sterility[a]		
Bacterial Endotoxins[a]		
Identity		
Vector DNA Identity		
Content		
[redacted]		
Biological Activity		
Potency[a]		
Infectivity[redacted]		
Purity		
[redacted]		

[redacted]

[Reviewer's note]

[redacted]

Validation of Analytical Procedures

Analytical methods have been validated in accordance with ICH guidelines or verified according to Ph. Eur./USP (compendial methods) as applicable.

[Reviewer's note]

[redacted]

Batch Analyses

Overview of Etranacogene Dezaparvovec Drug Product Batches

[redacted]

Batch Analyses Results for DP PV / PPQ Batches

[redacted]

Characterization of Impurities

All relevant data of process- and product-related impurities concerning DS and DP are described [redacted]. The product-related impurity profile for DP was consistent with the product-related impurity profile for DS.

No additional process-related impurities are expected to be present in the DP.

Product-related impurities that can possibly arise during the processing and storage of the DP are examined during release analytics and stability studies. Furthermore, a leachable and extractable study was performed.

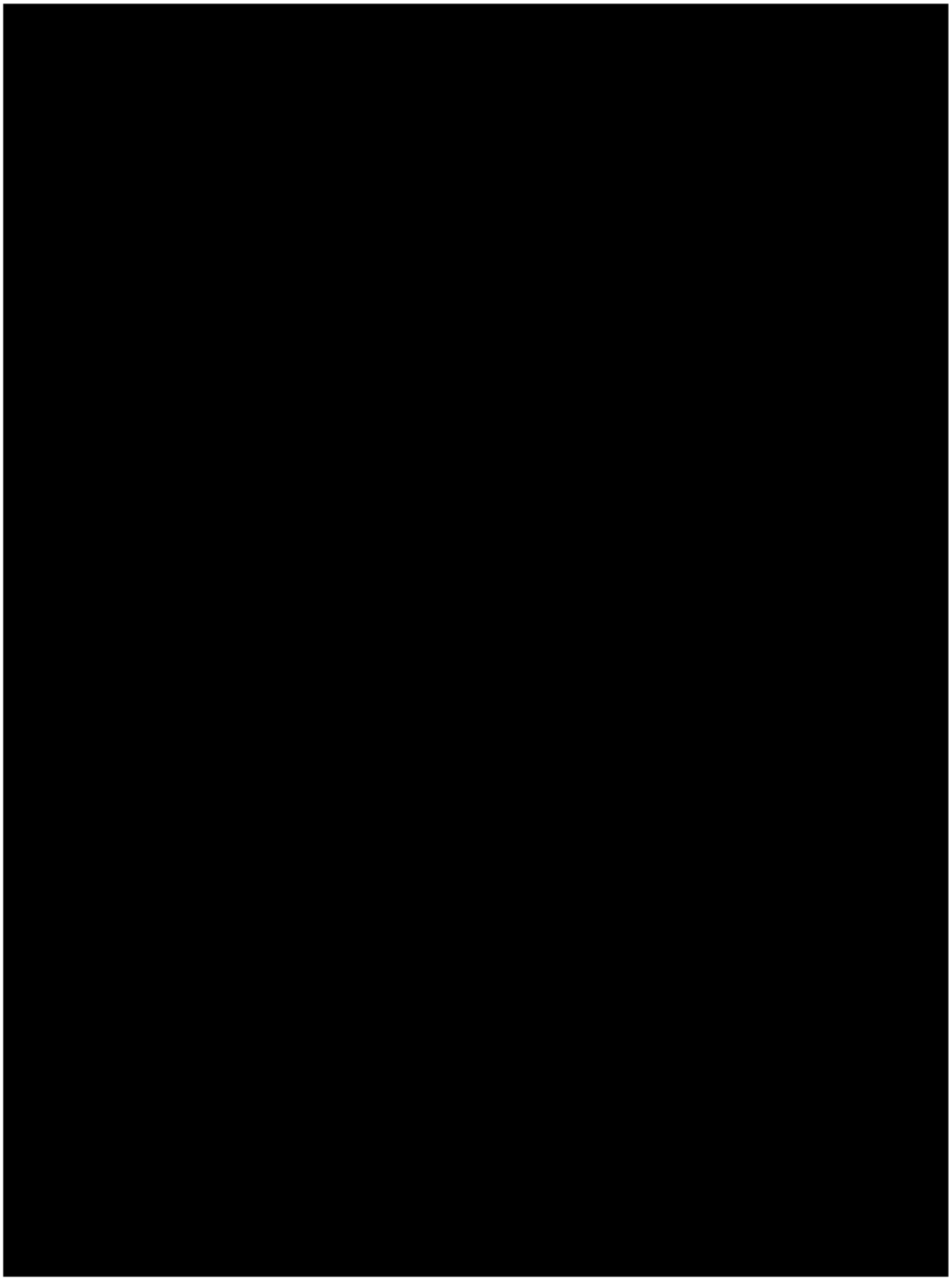
Justification of Specifications

The DP specifications were established based on a statistical analysis of historical manufacturing data [redacted] including assay capability, risks to process and/ or patients associated with variations within these stated product attributes, stability data as well as clinical experience. [redacted]

[redacted]

Justification of Specification (extract)

[redacted]



3.3.6 Reference Standards or Materials

This section describes the product derived, initial reference standard (IRS), primary reference standard (PRS), secondary reference standard (SRS), and where a standard may be used as a control sample (CS) for in-process, batch release and stability study testing for etranacogene dezaparvovec.

[redacted]

- Primary Reference Standard and Control Sample Used for Testing

  The current PRS [redacted] and control sample (CS; [redacted] used in the testing and release of DS and DP batches of etranacogene dezaparvovec and the respective tests where it is used [redacted]

  The development of the current PRS [redacted] is described. Qualification, re-qualification and storage conditions of reference standard are also described. [redacted]

[redacted]

[redacted]

- AMT-060 and Etranacogene Dezaparvovec Reference Material Development

  The development of product derived reference standard commenced with AMT-060 Phase I/II clinical trials and continued through Phase III clinical trial batches of etranacogene dezaparvovec. The development of AMT-060 reference standard and etranacogene dezaparvovec IRS and PRS are described.

  In addition to the development process, the bridging between standards, qualification, and approval for use in DS, DP and stability testing is described.

[redacted]

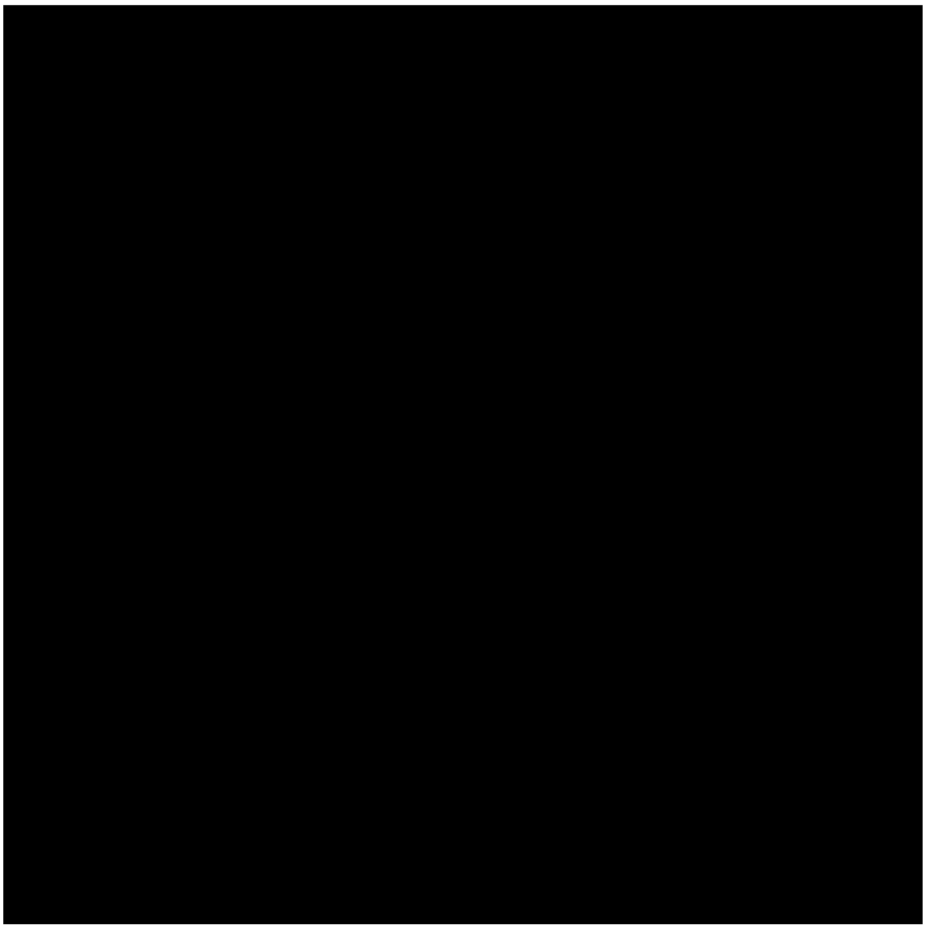
[redacted]

Standards are used to provide a qualitative or quantitative reference for the test batch, while CS are used as system suitability checks and to monitor for assay drift.

- Preparation and Qualification of Etranacogene Dezaparvovec Primary Reference Standard (PRS), [redacted]

[redacted]

- Criteria for Future Reference Materials – [redacted]



- Re-Qualification

  The requalification of product derived PRS and SRS used for testing the DS and DP are determined based on a [redacted] data review for each assay in which the reference standard is used. The scope of requalification is to confirm stability and therefore only a subset of tests from the release testing may be performed, based on the data review. [redacted]

3.3.7 Container Closure System

No novel components are utilized as a part of the container closure system.

(Materials included: Type I class glass, chlorobutyl rubber)	

Suitability for the Intended Application

A comprehensive evaluation of extractables and leachables (E&L) was performed for the etranacogene dezaparvovec DP. Additionally, the stoppers were subjected to the testing specified in USP <381> and <661>, and in Ph. Eur. 3.2.9, for the determination of the biological reactivity and extraction characteristics of elastomeric closures for containers.

Integrity testing of the container closure system is described

3.3.8 Drug Product Stability

Stability Conclusions

Based on the real-time stability data from the supporting studies performed on **batches** of DP **the proposed shelf-life for DP stored at +5°C ± 3°C is**

[Reviewer's note]

Please update the long-term stability test results of DP.

- The sponsor updates the long-term stability test results of DP .
  - **According the real-time long-term stability data, the shelf-life for DP stored at +5°C ± 3°C is 18 months and protected from light**

Drug Product Stability Batches *(The stability data has been updated after request)*

[redacted]

Container Closure System

[redacted] All stability samples were stored protected from light [redacted]

Stability Data

- Long-Term Stability Data ([redacted]

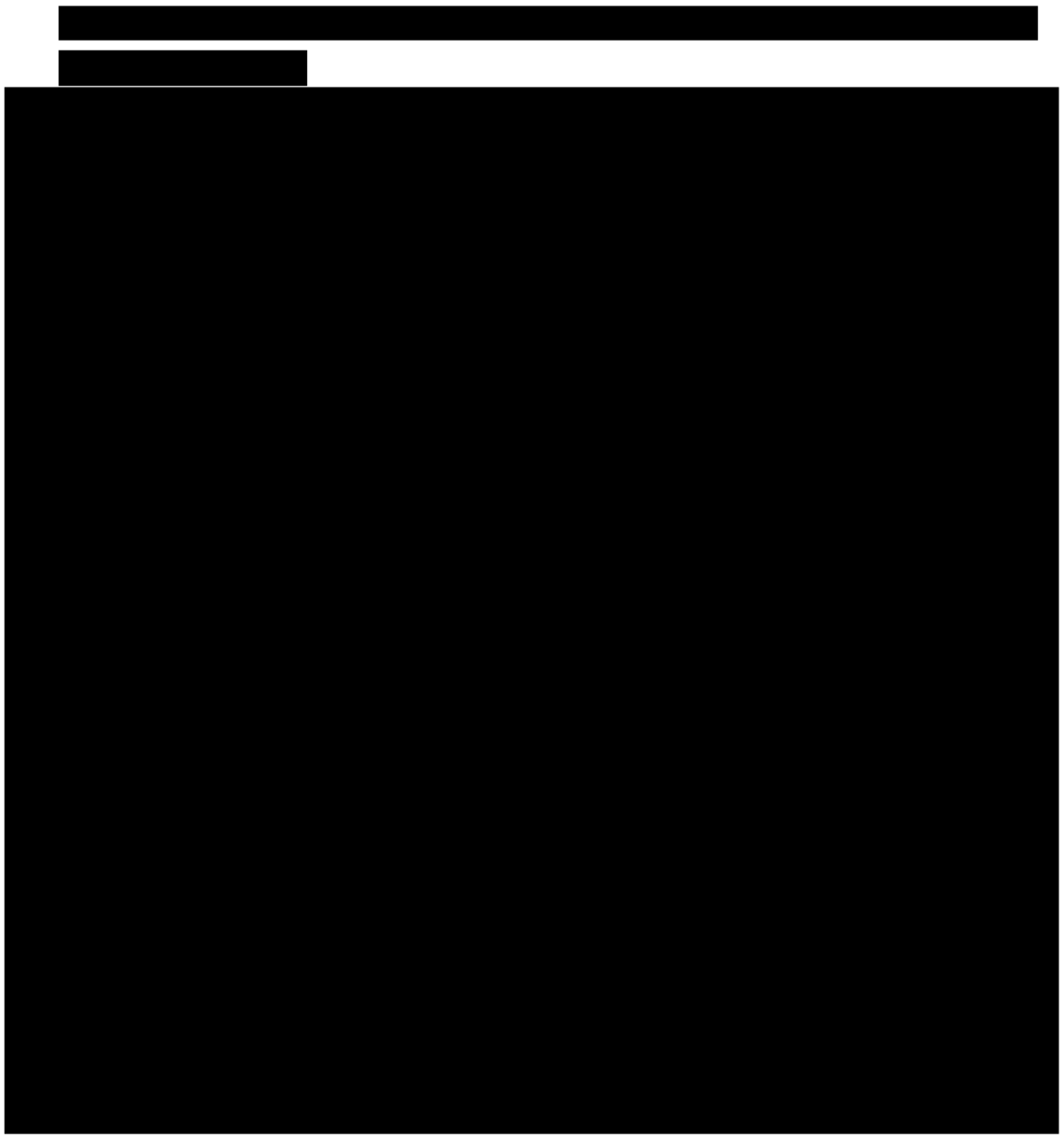
  (The stability data has been updated after request)

  [redacted]

[redacted]

[Reviewer's note]

- The sponsor's evaluation and disposition are acceptable.



[redacted]

[redacted]

[redacted]

[Reviewer's note]

[redacted]

- [redacted] the shelf-life for DP stored at +5°C ± 3°C is 18 months and protected from light.

- Accelerated Stability Data [redacted]

[redacted]

[redacted]

[redacted]

- Forced Degradation

Forced degradation studies were performed on the DP [redacted] to evaluate the stability-indicating capability of the analytical methods. The resulting samples were analyzed [redacted] The results support the stability-indicating capability of the [redacted] assays.

- Photostability

A photostability study was performed on DP [redacted] to determine if the product is light-sensitive. The resulting samples were analyzed [redacted]. **The results indicated that the DP is light-sensitive in primary packaging. The proposed secondary packaging for commercial supplies is effective in protecting the DP from photodegradation as it is also light obscure packaging**. Based on the results of the photostability study performed according to ICH Q1B, **the labeling of DP recommends protecting the product from light.** In-use stability studies of DP after dilution for up to 24 hours at room temperature when protected from light are discussed [redacted]

A.2

[redacted]

[redacted]

[redacted]

Viral Adventitious Agents – Virus Risk Assessments

- AcNPV Risk Assessment

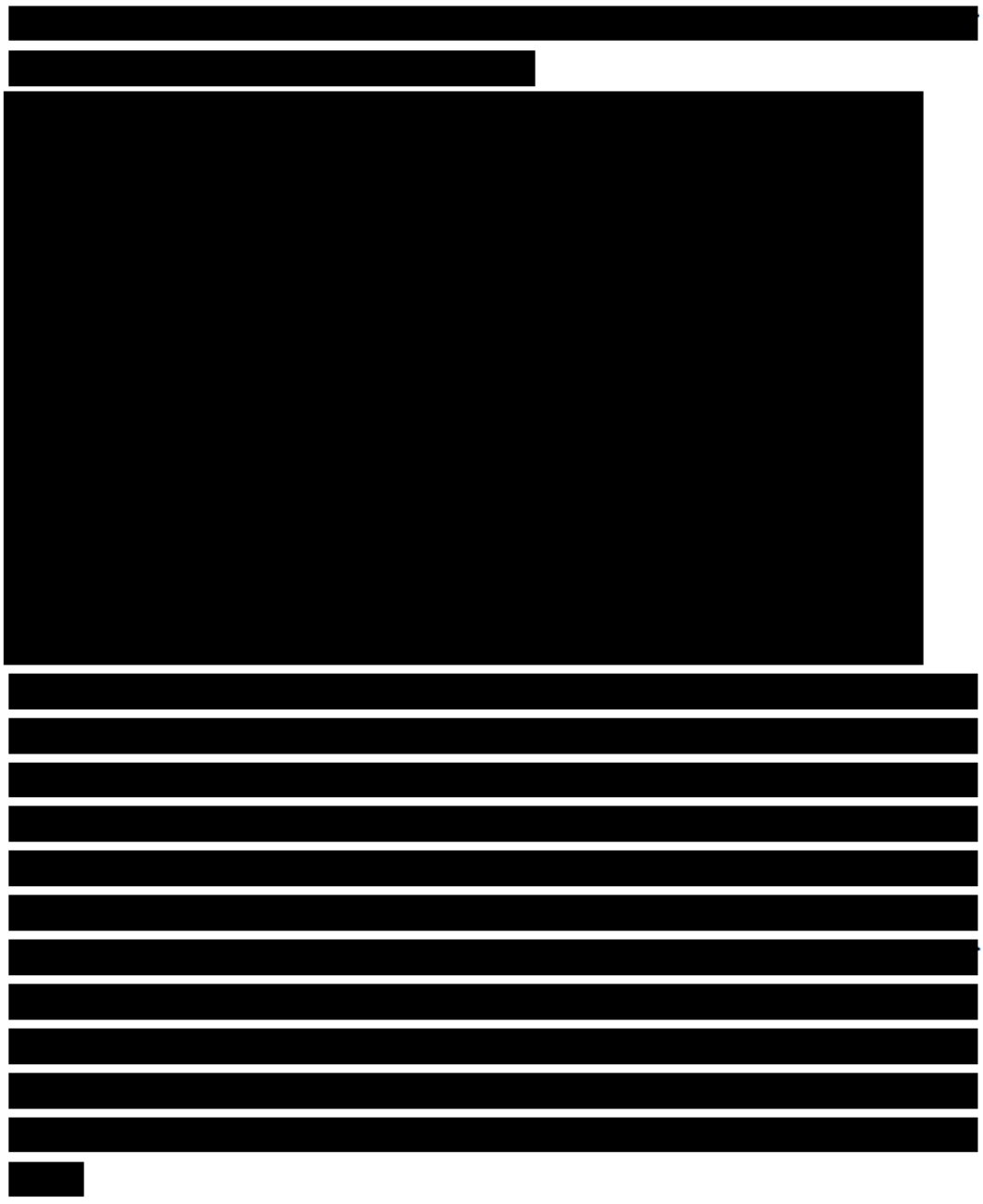
  [redacted]

  [redacted]

- Sf Rhabdovirus Risk Assessment

  [redacted]



3.4 External Communication (deficiency/inquiry and supplement, expert consultation…etc.)

The applicant's responses to the supplementary information have been included in the relevant chapters of the report.

3.5 Conclusion and Recommendation

From the CMC perspective, Hemgenix (Etranacogene dezaparvovec) is recommended to be approved. The stability results support the shelf life of the drug product protected from light at 5 ± 3°C for 18 months.

4. Non-clinical Pharmacology/Toxicology Evaluation

4.1. Introduction

This NDA of **Hemgenix®** [Etranacogene dezaparvovec/AAV5-hFIXco-Padua/AMT-061 (non-clinical: AMT-060), $1x10^{13}$ genome copies (gc)/mL, suspension for intravenous infusion] is filed by CSL Behring (傑特貝林有限公司). **Hemgenix®** is indicated for the treatment of adults with Hemophilia B (congenital factor IX deficiency). **Hemgenix®** is administered via intravenous infusion as a single dose of $2x10^{13}$ gc per kilogram (kg) of body weight (BW).

Hemophilia B [factor IX (FIX) deficiency], caused by a defect in the *F9* gene, is an X-linked inherited coagulation factor deficiency that predominantly affects males and results in lifelong bleeding disorders. Females who are heterozygous carriers can also be affected and may experience occasional mild bleeding symptoms.

Etranacogene dezaparvovec is an adeno-associated virus (AAV) vector-based gene therapy designed for the long-term expression of functional human FIX in the liver for the long-term treatment of hemophilia B. It is a recombinant adeno-associated virus 5 (AAV5) vector containing an expression cassette encoding a codon-optimized DNA sequence for the Padua-variant of the human FIX (hFIXco-Padua) under the control of a liver-specific promoter (LP1) [redacted]

[redacted]

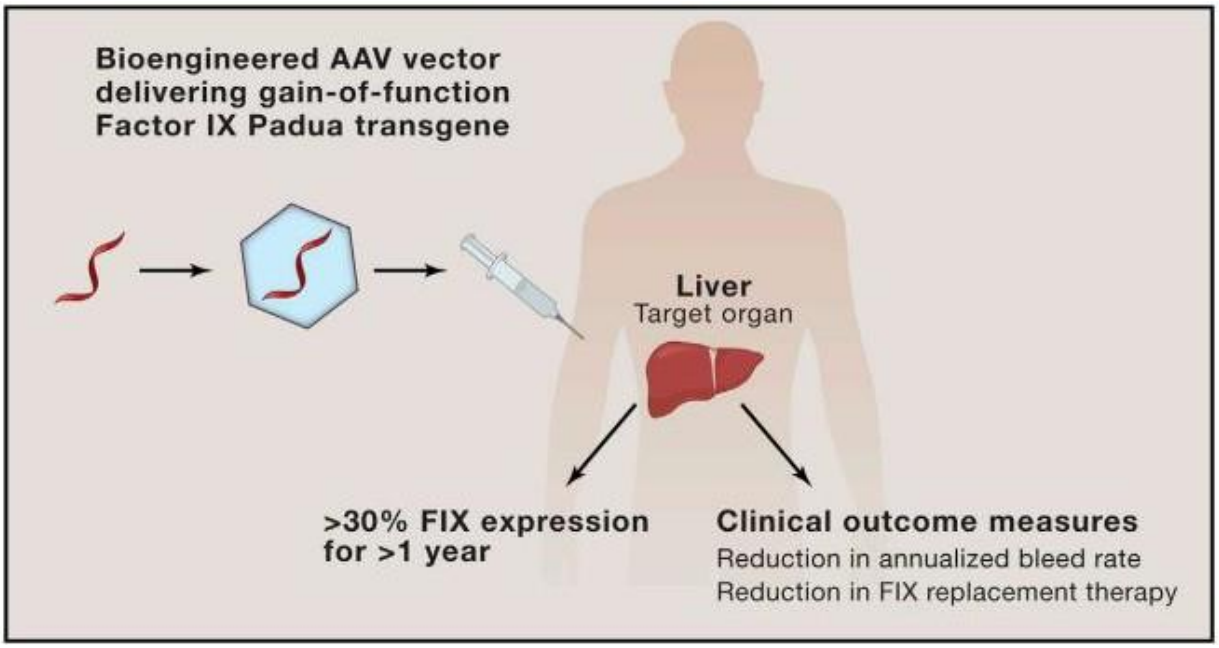
A) The expression cassette is flanked by two inverted terminal repeats (hairpin structures) and consists of the Liver-specific promoter 1 (LP1), Simian virus 40 (SV40)-intron, hFIXco-Padua coding sequence, and SV40 late-poly(A) signal.
B) PP = Pre-pro-peptide; AP = Activation peptide

[redacted]

4.2. Pharmacology

4.2.1. Mechanism of Action

Etranacogene dezaparvovec is a recombinant AAV5 vector containing a transgene encoding the gain-of-function Padua-variant (R338L) of human FIX (hFIXco-Padua) under the control of LP1 promoter. Following IV infusion, etranacogene dezaparvovec transduces liver cells, resulting in LP1 promoter-directed long-term expression of FIX-Padua, and consequently increased circulating FIX activity in patients with hemophilia B.



(Figure excerpted from Cell 171(7):1478-1480, 2017)

Figure 2 Schematic Representation of the Mechanism of Action of Etranacogene Dezaparvovec

4.2.2. Primary Pharmacodynamics

The non-clinical studies were first conducted with AMT-060, which encodes wild-type (WT) human FIX, and then with etranacogene dezaparvovec (AMT-061), which encodes human FIX-Padua. The vector genome of AMT-060 and etranacogene dezaparvovec are identical, except for the 2-nucleotide change in the FIX coding sequence resulting in a single amino acid substitution.

4.2.2.1. *In Vitro* Studies

No in vitro pharmacology studies have been performed.

4.2.2.2. *In Vivo* Studies

Studies/Species	Dose/Route	Noteworthy Findings
Biological activity of AMT-060 assessed in C57Bl/6 mice (Proof-of-Concept) WT mouse [NR-060-11-007]	AMT-060 0, $1x10^{12}$, $2.5x10^{12}$, $5x10^{12}$ gc/kg Single dose i.v. infusion Vehicle: PBS + 5% sucrose	• AMT-060 showed effective delivery of vector DNA to the liver, hFIX mRNA expression in the liver, and measurable plasma hFIX protein in a vector dose-dependent manner. • Plasma hFIX protein levels were measured up to 10% of normal at 4 weeks post-infusion at $5x10^{12}$ gc/kg of AMT-060.
Biological Activity of AAV5-hFIX (AMT-060) in an Animal Pharmacological Model of	AMT-060 0, $5x10^{11}$, $5x10^{13}$, $2.3x10^{14}$ gc/kg	• Administration of AMT-060 resulted in a dose-dependent increase of plasma FIX protein levels (ranging from 1-5%, 350-1700%, 1300-5900% of normal human levels in LD, MD, and HD, respectively) and the associated plasma FIX

Hemophilia B (Proof-of-Concept) Hemophilia B mouse (FIX knockout) [NR-060-13-007]	Single dose i.v. Vehicle: PBS + 5% sucrose	activity (aPTT assay; total FIX clotting activity) in hemophilia B mice.
AMT-060: Investigative Study of Single-dose Administration to Newborn, Juvenile and Adult Mice followed by a Long-term Observation Period WT mouse (neonate, weanling juvenile, young adult, adult) [NR-060-14-008]	AMT-060 0, $2.3x10^{14}$ gc/kg Single dose i.v. Vehicle: PBS + 5% sucrose	• All TA-treated animals showed sustained plasma hFIX protein expression at all sampling timepoints from 4 weeks post-dosing until 18 months of age (lower levels in neonatal mice and weanlings).
Pharmacodynamics of AMT-060 in Biodistribution and Toxicity Study in Mice WT mouse [NR-060-14-002] [NR-060-13-006]	AMT-060 0, $5x10^{11}$, $5x10^{13}$, $2.3x10^{14}$, $2.3x10^{14}$+prednisone # gc/kg Single dose i.v. Vehicle: PBS/5% sucrose #1 mg/kg, TIW	• Administration of AMT-060 resulted in dose-dependent plasma hFIX protein levels, ranging from 0.2 to 6267% of normal human FIX levels at day 28. • HD±prednisone showed a peak in hFIX protein level at 4 weeks followed by a decline of hFIX protein level at later timepoints. HD+prednisone showed lower levels of hFIX expression compared to HD group, while the vector DNA levels were comparable.
Pharmacodynamics of Etranacogene Dezaparvovec and AMT-060 in Biodistribution and Toxicity Study in Mice WT mouse [NR-061-18-002]	**etranacogene dezaparvovec** or AMT-060 0, $5x10^{11}$, $5x10^{12}$, $5x10^{13}$, $5x10^{13}$-PS-20## gc/kg Single dose i.v. Vehicle: PBS/5% sucrose/0.02% PS-20 (clinical formulation) ##formulated without PS-20	• Both AMT-060 and etranacogene dezaparvovec showed comparable, vector dose-dependent plasma hFIX protein levels and a dose-response in FIX activity (chromogenic and aPTT assays). • The FIX activity in etranacogene dezaparvovec-treated animals at MD and HD was 3- to 4-fold (chromogenic) or 5- to 6-fold (aPTT) higher, respectively, when compared to AMT-060. • No difference in plasma hFIX protein and FIX activity was observed between formulations ± PS-20.
Dose Efficacy Testing in Mice of 5 Different Batches of Etranacogene Dezaparvovec with a Range of in Vitro Potencies WT mouse [NC-RPT-00006]	**etranacogene dezaparvovec w/ different RU** 0, $5x10^{11}$, $5x10^{12}$, $5x10^{13}$ gc/kg Single dose i.v. Vehicle: PBS/5% sucrose/0.02% PS-20	• In vitro potency of the 5 batches in HuH-7 hepatocellular carcinoma cell line was ranged from 0.4-1.9 RU. • Batches with different in vitro potencies resulted in comparable liver transduction (qPCR), hFIX protein expression (ELISA), and FIX activity (chromogenic) in mice at each dose level tested.
A Single Dose Study of AAV5-LP1-hFIXco vector by Intravenous Injection in Rhesus Monkeys with a 90-Day Recovery Period - In-house analysis of vector	AMT-060 $5x10^{12}$ gc/kg (8 mL/kg) Single dose i.v. infusion	• Administration of AMT-060 resulted in delivery of vector DNA to the liver [redacted] hFIX mRNA expression in the liver [redacted] and measurable plasma hFIX protein (up to 18% 1 week post-doing and stabilized around 5% of normal human levels for at least 13 weeks; by ELISA) 13 weeks after dosing.

DNA delivery and transgene expression (Proof-of-Concept) Rhesus macaques monkey [NR-060-12-003] [NR-060-11-009]	Vehicle: PBS + 5% sucrose	
Pharmacodynamics of AMT-060 in Biodistribution and Toxicity Study in Cynomolgus Macaques Cynomolgus macaques monkey [NR-060-14-010] [NR-060-14-006] [NR-060-14-011]	AMT-060 $5x10^{11}$, $5x10^{12}$, $2.5x10^{13}$, $9.3x10^{13}$ gc/kg Single dose i.v. infusion Vehicle: 0.9% w/v sodium chloride	• Single-dose IV administration of AMT-060 in cynomolgus macaques showed a dose-response relationship in plasma hFIX protein levels, with peak levels ranging from 0.5% - 30% and stabilized thereafter at 0.3% - 15% of normal human levels. • The pre-existing anti-AAV5 antibodies in serum before dosing did not show a notable effect on vector DNA levels or plasma hFIX protein levels after dosing of AMT-060. • The presence of hFIX-specific antibodies in HD group animals following administration of AMT-060 was associated with decreased or loss of plasma hFIX protein after having shown peak levels.
Pharmacodynamics of etranacogene dezaparvovec and AMT-060 in Biodistribution and Toxicity Study in Cynomolgus Macaques Cynomolgus macaques monkey [NR-061-17-001]	**etranacogene dezaparvovec** 0, $5x10^{12}$, $2.5x10^{13}$, $5x10^{11}$###, $9x10^{13}$ or AMT-060 $5x10^{12}$ gc/kg Single dose i.v. Vehicle: PBS/5% sucrose ###added later, sacrificed together	• Single-dose IV infusion of etranacogene dezaparvovec in cynomolgus macaques showed similar kinetics of hFIX protein expression with those observed in the study with AMT-60, in which a dose-response relationship in plasma hFIX protein levels was demonstrated, with peak levels ranged from 4.2% - 37% and stable levels from week 4 to week 13 at 1.3% - 52% of normal human levels. • The kinetics of hFIX protein expression were comparable for etranacogene dezaparvovec and AMT-060 at LD. • A dose-response relationship in FIX activity (chromogenic and aPTT) was observed in etranacogene dezaparvovec-treated animals, reaching 350% (chromogenic) and >400% (aPTT) of normal human levels in HD. At equal doses, the hFIX activity in etranacogene dezaparvovec-treated animals was 6-fold (aPTT) higher than in AMT-060-treated animals. • The decreased hFIX protein levels and associated FIX activity observed in individual animals were attributed to the developed anti-hFIX Ab.

LD: low dose; MD: medium dose; HD: high dose; TA: test article; WT: wild-type

4.2.2.3. Primary Pharmacology Summary:

- In wild-type mice and in the mouse model of hemophilia B, IV administration of AMT-060 at doses up to 5 $x10^{12}$ and $2.3x10^{14}$ gc/kg, respectively, resulted in dose-dependent increases in vector transduction in the liver, plasma hFIX protein levels and plasma hFIX activity.
- Following receiving a single dose of AMT-060 at $2.3x10^{14}$ gc/kg in mice at different ages, sustained plasma hFIX protein expression at all sampling timepoints from 4 weeks post-dosing until 18 months of age was observed. Lower levels of hFIX protein were observed in neonatal mice and weanlings.
- In mice, IV administration of AMT-060 or etranacogene dezaparvovec at doses up to 5 $x10^{13}$ gc/kg resulted in comparable, dose-dependent plasma hFIX protein levels and a dose-response in FIX activity. Etranacogene dezaparvovec-treated animals showed higher hFIX chromogenic and clotting activity compared to mice receiving AMT-060.

- In mice, batches of etranacogene dezaparvovec with different in vitro potencies revealed comparable liver transduction, hFIX protein expression, and FIX activity levels.
- Similarly, in non-human primates (NHPs), single-dose IV administration of etranacogene dezaparvovec at doses of $5x10^{12}$ to $9x10^{13}$ gc/kg also showed a dose-response relationship in plasma hFIX protein levels, with peak levels ranging from 4.2% - 37% and stable levels from week 4 to week 13 at 1.3% - 52% of normal human levels. A dose-response relationship in FIX chromogenic and clotting activity was observed in etranacogene dezaparvovec-treated animals, reaching more than 400% of normal human levels in animals receiving $9x10^{13}$ gc/kg. At equal doses, the hFIX clotting activity in etranacogene dezaparvovec-treated animals was 6-fold higher than in AMT-060-treated animals.
- In NHPs, the decreased hFIX protein levels and associated FIX activity observed in individual animals were attributed to the developed anti-hFIX Ab.

4.2.3. Secondary Pharmacodynamic

No secondary pharmacodynamic studies were conducted.

4.2.4. Safety Pharmacology

No standalone safety pharmacology studies were conducted. Safety pharmacology endpoints including clinical observations and ECG were assessed in the GLP toxicity studies of AMT-060 or etranacogene dezaparvovec and no effects were found.

4.2.4.1. Safety pharmacology summary:

- No effects on safety pharmacology endpoints including clinical observations and ECG were noted in the GLP toxicity studies of AMT-060 or etranacogene dezaparvovec.

4.2.5. Pharmacokinetics

No standalone pharmacokinetic studies were conducted. Pharmacokinetic assessments of etranacogene dezaparvovec and AMT-060, including biodistribution, shedding profile, and germline transmission were incorporated in pharmacodynamic and toxicity studies in mice and NHPs.

Studies/Species	Dose/Route	Noteworthy Findings
Biodistribution in mice (qPCR)		
Biological activity of AMT-060 assessed in C57Bl/6 mice (Proof-of-Concept) WT mouse [NR-060-11-007]	AMT-060 0, $1x10^{12}$, $2.5x10^{12}$, $5x10^{12}$ gc/kg Single dose i.v. infusion Vehicle: PBS + 5% sucrose	• IV single-dose administration of AMT-060 delivered the vector DNA to the liver ($9.4x10^3$, $2x10^4$, $8x10^4$ gc/mcg DNA for LD, MD, HD, respectively) in a vector dose-dependent manner.

AMT-060: Investigative Study of Single-dose Administration to Newborn, Juvenile and Adult Mice followed by a Long-term Observation Period WT mouse (neonate, weanling juvenile, young adult, adult) [NR-060-14-008]	AMT-060 0, $2.3x10^{14}$ gc/kg Single dose i.v. Vehicle: PBS + 5% sucrose	• At 4 weeks post-dosing, animals at ages ≥ 6 weeks showed similar vector NDA levels [redacted] in the livers, while the levels in animals at ages ≤ 3 weeks were 1-2 log lower than those observed in the older mice. • At 18 months of age, the vector DNA levels in the liver were one log lower ([redacted] than the levels at 4-week post-doing, except a 2 log lower levels [redacted]) observed in animals dosed at age of day 2. • Long-term persistence of vector DNA in the liver up to 18 months post-dosing was demonstrated in mice dosing at different ages
Biodistribution and Toxicity Studies with AMT-060 in Mice WT mouse [NR-060-14-002] [NR-060-13-006]	AMT-060 0, $5x10^{11}$, $5x10^{13}$, $2.3x10^{14}$, $2.3x10^{14}$+prednisone # gc/kg Single dose i.v. Vehicle: PBS/5% sucrose #1 mg/kg, TIW	• In animals treated with HD±prednisone, vector DNA (qPCR) was detected in all samples collected on day 8 and day 180; the vector DNA profile was comparable between groups treated with or without prednisone. • The vector DNA levels were consistently lower at day 180 when compared to day 8 in all tissues • The highest vector DNA level on both day 8 and day 180 was detected in the liver with at least one log higher than in other tissues.
Biodistribution and Toxicity Studies with Etranacogene Dezaparvovec and AMT-060 in Mice WT mouse [NR-061-18-002]	**etranacogene dezaparvovec** or AMT-060 0, $5x10^{11}$, $5x10^{12}$, $5x10^{13}$, $5x10^{13}$-PS-20## gc/kg Single dose i.v. Vehicle: PBS/5% sucrose/0.02% PS-20 (clinical formulation) ##formulated without PS-20	• IV administration of etranacogene dezaparvovec and AMT-060 in mice resulted in a similar profile of vector DNA in the plasma, liver and extrahepatic tissues, regardless of the formulation. Plasma vector DNA levels peaked on day 1 [redacted]) and gradually decreased [redacted] by week 13. In the liver, a dose-dependent increase in vector DNA was shown. • At 13 weeks post-dosing, vector DNA was detected in all tissues in animals receiving etranacogene dezaparvovec at HD, with the highest level in the liver [redacted] followed by adrenal glands and lowest in bone marrow [redacted] In extrahepatic tissues, except for the adrenal glands, vector DNA levels were approx. 100-fold lower than in the liver.
Biodistribution in NHP (qPCR)		
A Single Dose Study of AAV5-LP1-hFIXco vector by Intravenous Injection in Rhesus Monkeys with a 90-Day Recovery Period - In-house analysis of vector DNA delivery and transgene expression (Proof-of-Concept) Rhesus macaques monkey [NR-060-12-003] [NR-060-11-009]	AMT-060 $5x10^{12}$ gc/kg (8 mL/kg) Single dose i.v. infusion Vehicle: PBS + 5% sucrose	• At 13 weeks post-dosing, single dose IV infusion of AMT-060 at $5x10^{12}$ gc/kg demonstrated a homogenous spread of vector DNA among the liver lobes to a comparable level between animals [redacted]
Biodistribution and Toxicity Studies with AMT-060 in Cynomolgus Macaques	AMT-060 $5x10^{11}$, $5x10^{12}$, $2.5x10^{13}$, $9.3x10^{13}$ gc/kg Single dose i.v. infusion	• IV administration of $5x10^{11}$ to $9.3x10^{13}$ gc/kg AMT-060 in NHPs, the levels of vector DNA in the serum time-dependently declined over the 26-week observation period. The vector DNA levels in saliva and urine were multiple logs lower than in serum and followed a similar clearance profile for serum.

Cynomolgus macaques monkey [NR-060-14-010] [NR-060-14-006] [NR-060-14-011]	Vehicle: 0.9% w/v sodium chloride	• Vector DNA was undetectable in saliva from weeks 8 to 12 and in urine around week 8. • Measurable, trace, or low amounts of vector DNA could be detected in semen collected from animals receiving ≥ $2.5x10^{13}$ gc/kg. • At 26 weeks post-dosing, single dose IV infusion of AMT-060 at doses ≥ $5x10^{12}$ gc/kg resulted in homogenous vector DNA distribution in the liver to a comparable level between animals. • In animals treated with AMT-060, vector DNA was detectable in all tissues in a dose-dependent manner. The highest levels were found in the liver and adrenal glands, followed by the spleen. The levels in all other tissues were approximately 10- to 100-fold lower than those in the liver. Vector DNA was detected in the testis, epididymis, and seminal vesicles [redacted] in animals receiving HD. • hFIX mRNA was only quantifiable in the liver.
Biodistribution and Toxicity Study with Etranacogene Dezaparvovec and AMT-060 in Cynomolgus Macaques Cynomolgus macaques monkey [NR-061-17-001]	**etranacogene dezaparvovec** 0, $5x10^{12}$, $2.5x10^{13}$, $5x10^{11}$###, $9x10^{13}$ or AMT-060 $5x10^{12}$ gc/kg Single dose i.v. Vehicle: PBS/5% sucrose ###added later, sacrificed together	• IV administration of etranacogene dezaparvovec and AMT-060 in NHPs resulted in a similar profile of vector DNA in the plasma (comparable AUC), liver and extrahepatic tissues. The average hFIX mRNA levels (RT-qPCR) were also comparable for AMT-060 [redacted] and etranacogene dezaparvovec [redacted] at an equal dose of $5x10^{12}$ gc/kg. • Etranacogene dezaparvovec vector DNA levels in plasma peaked on day 1, declined subsequently, and detectable at 6 months post-dosing at all doses except for the lowest dose. Vector DNA in urine was undetectable at 3 months post-dosing. • At 26 weeks post-dosing, vector DNA was detected in all tissues (including testis, epididymis, and seminal vesicles), in a dose-dependent manner in animals receiving $5x10^{12}$ to $9x10^{13}$ gc/kg of etranacogene dezaparvovec, with highest level in the liver ([redacted] followed by adrenal glands [redacted] and spinal cord [redacted] In the rest of the extrahepatic tissues, vector DNA levels were $<5x10^{5}$ gc/mcg DNA. Quantifiable hFIX mRNA was primarily detected in the liver and also detected at levels close to the LLOQ in the adrenals, spleen, kidney, spinal cord, and heart in some animals treated with higher or the highest doses. • Transduction of liver cells was assessed by FISH and revealed a dose-related increase in cell transduction [redacted] At equal dose, transduction mediated by AMT-060 and etranacogene dezaparvovec was comparable [redacted]
Paternal germline transmission (to untreated females and their offspring)		
AMT-060 Paternal Germline Transmission in C57Bl/6 Mice (Biodistribution) WT mouse [NR-060-14-001]	AMT-060 0, $2.3x10^{14}$ gc/kg Single dose i.v. ♂: -d6 prior mating ♀: untreated Vehicle: PBS + 5% sucrose	• In the AMT-060-treated male mice, vector DNA was detected in the male reproductive tissues, including epididymis, seminal vesicle, sperm, and testes. Following mating with treated males, vector DNA was not detected in the fetus and female reproductive tissues of the untreated female mice, including the uterus and placenta.

LD: low dose; MD: medium dose; HD: high dose; TA: test article; WT: wild-type

4.2.5.1. Pharmacokinetics summary:

- In mice and NHPs, IV administration of etranacogene dezaparvovec and AMT-060 resulted in a similar profile of vector DNA in the plasma, liver, and extrahepatic tissues. Long-term persistence of vector DNA in the liver up to 18 months post-dosing was demonstrated in mice dosing at different ages.
- At 13 weeks post-dosing in mice receiving $5x10^{13}$ gc/kg of etranacogene dezaparvovec, plasma vector DNA levels peaked on day 1 and gradually decreased by the end of the study. Vector DNA was detected in all tissues examined, with the highest level in the liver, followed by adrenal glands, and lowest in bone marrow.
- In NHPs, the kinetics of vector DNA in the serum were similar to those observed in mice. The AMT-060 vector DNA levels in saliva and urine were multiple logs lower than in serum and followed a similar clearance profile for serum. Measurable, trace, or low amounts of vector DNA could be detected in semen collected from animals receiving $\geq 2.5x10^{13}$ gc/kg of AMT-060.
- At 26 weeks post-dosing in NHPs receiving $5x10^{12}$ to $9x10^{13}$ gc/kg of etranacogene dezaparvovec, vector DNA was detected in all tissues (including testis, epididymis and seminal vesicles), in a dose-dependent manner, with highest level in the liver, followed by adrenal glands and spinal cord. In the rest of the extrahepatic tissues, vector DNA levels were 1-2 log lower than in liver. Quantifiable hFIX mRNA was primarily detected in the liver and also detected at levels close to the LLOQ in the adrenals, spleen, kidney, spinal cord, and heart in some animals treated with higher or the highest doses. A dose-related increase in cell transduction percentage of 17% up to 46% was determined by FISH.
- In a paternal germline transmission study with AMT-060 in mice, vector DNA was detected in the male reproductive tissues in the AMT-060-treated male mice, but not detected in the fetus and female reproductive tissues of the untreated female mice.

4.3. Toxicology

4.3.1. Single-dose/Repeated-dose Toxicity Study

Studies/Species/	Dose/Route	Noteworthy Findings
6-month observation Mouse (90-114♂/group) Initial age: 8~13 weeks old [NR-060-14-002] GLP: Yes TF: CRL 1st dosing: N/A SFs: safety of co-treatment with prednisone, BD of the vector, hFIX protein analysis	AMT-060 0, $5x10^{11}$, $5x10^{13}$, **$2.3x10^{14}$**, $2.3x10^{14}$+prednisone # gc/kg Single dose i.v. Vehicle: PBS/5% sucrose	▪ Parameters: Mortality, CS, BW, FC, OP, inflammation markers Serum Amyloid A & IL-6 (d8), Hema, CC, OW, Macro, Micro (additional exam of liver only: 20 mice/group at d28) ▪ 4 unscheduled deaths (1LD, 1HD, 2HD+prednisone; not TA-related) TA-related ▪ **[OW]:** ↑spleen (HD), assoc. w/ Micro; ↓thymus (HD+ prednisone), assoc. w/ Micro

	#1mg/kg prednisone (p.o.), from -1 week ~ study end, TIW	▪ **[Micro:\spleen]:** ++/+++ hypercellularity in the germinal centers (HD, d8, transient; ↓incidence and severity by co-Tx w/ prednisone), **[:\thymus]**: +atrophy (HD+ prednisone) ▪ NOAEL= $2.3x10^{14}$ gc/kg
13-week observation Mouse (16♂/group) Initial age: 7~8 weeks old [NR-061-18-002] GLP: Yes TF: CRL 1st dosing: N/A SFs: comparability of the safety of etranacogene dezaparvovec and AMT-060	**Etranacogene dezaparvovec** or AMT-060 0, $5x10^{11}$, $5x10^{12}$, **$5x10^{13}$**, $5x10^{13}$ (-PS-20) gc/kg Single dose i.v. Vehicle: PBS/5% sucrose/0.02% PS-20 (clinical formulation)	▪ Parameters: Mortality, CS, BW, Hema, CC, OW, Macro, Micro (major tissues) ▪ 2 unscheduled deaths (1C, 1LD, not TA-related) ▪ No TA-related effects: mortality, CS, BW, Hema, CC TA-related ▪ **[Micro:\lung]:** + pulmonary thrombi (1HD/etranacogene dezaparvovec, 1HD/AMT-060/-PS-20), non-adverse (low incidence, minor severity, not found at the higher dose in the other study) ▪ NOAEL= $5x10^{13}$ gc/kg
6-month observation Monkey (3♂/group)## Initial age: 31~45 months [NR-060-14-010] GLP: Yes TF: CRL 1st dosing: N/A SFs: BD and shedding of the vector, hFIX protein analysis	AMT-060 $5x10^{11}$, $5x10^{12}$, $2.5x10^{13}$, **$9.3x10^{13}$** gc/kg Single dose i.v. infusion Vehicle: 0.9% w/v sodium chloride	▪ Parameters: Mortality, CS, BW, ECG, BT, Hema, Coagulation, CC, inflammatory markers, UA, OW, Macro, Micro ▪ ##Only animals w/o pre-existing anti-AAV5 capsid titers were included in the study ▪ No TA-related effects on the parameters assessed ▪ NOAEL= $9x10^{13}$ gc/kg
$13^{###}$- or 26-week observation Monkey (3♂/group) Initial age: 26~30 months [NR-061-17-001] GLP: Yes TF: Labcorp 1st dosing: N/A SFs: BD and shedding of the vector, hFIX protein analysis	**Etranacogene dezaparvovec** 0, $5x10^{12}$, $2.5x10^{13}$, $5x10^{11###}$, **$9x10^{13}$** or AMT-060 $5x10^{12}$ gc/kg Single dose i.v. Vehicle: PBS/5% sucrose ###added later, sacrificed together	▪ Parameters: Mortality, CS, BW, FC, ECG, Hema, Coagulation, CC, additional liver enzymes, thrombin-antithrombin complex (TAT), D-dimer, anti-AAV5 capsid, OW, Macro, Micro ▪ No TA-related effects: mortality, CS, TAT, D-dimer, CS, BW, ECG, Hema, CC, OW, Macro, Micro, safety pharmacology endpoints (CNS and cardiovascular system, lung and kidney function) TA-related ▪ **[liver enzyme]:** ++↑AST (up to 4.5X), ALT (up to 3.0X), transient (d2, 4); ▪ ↑high titer against AAV5 capsid (6-mo); ▪ **[Coagulation]:** ↓aPTT w/ ++↑PT (HD), not assoc. w/ ↑coagulation or higher thrombin utilization ▪ NOAEL= $9x10^{13}$ gc/kg

CS: Clinical Sign; BW: Body Weight; FC: Food Consumption; OP: Ophthalmology; ECG: Electrocardiography; Hema: Hematology; CC: Clinical Chemistry Urin: Urinalysis; OW: Organ Weight; Macro: Macroscopy; Micro: Microscopy; TK: Toxicokinetics; OT: others.
+: minimal; ++: mild or slight; +++: moderate; ++++: marked or severe; +++++: very severe or massive
LD: low dose; MD: medium dose; HD: high dose; TA: test article; BD: biodistribution

4.3.2.1. Toxicology summary:

- GLP single-dose IV toxicity studies of AMT-060 or etranacogene dezaparvovec at doses up to10- and 5-fold the recommended human dose of $2x10^{13}$ gc/kg in mice and monkeys, respectively, with an observation period of up to 6 months, were generally well-tolerated. No AMT-060- or etranacogene dezaparvovec-related adverse effects were observed except for non-adverse effects such as minimal pulmonary thrombi observed in two mice treated at the highest dose, and a transient mild elevation of liver enzymes.

- The anti-drug antibodies against the AAV5 vector could be observed in NHPs at the end of the 6-month observation period.

4.3.2. Local Tolerance

Local tolerance at the injection site has been assessed as part of the GLP general toxicity studies. No notable findings were observed at the injection site.

4.3.3. Genotoxicity

Evaluation of genotoxic risk was based on integration site analysis, i.e., analysis of insertion of vector DNA sequence in genomic DNA isolated from livers of mice and NHPs treated with AMT-060, coupled with bioinformatical data mining.

[redacted]

4.3.3.1. Genotoxicity Summary:

- In an integration site analysis study conducted with liver tissues collected 6 months after infusion, from mice and NHPs receiving AMT-060, a measurable level of integration of vector DNA in both species was found. The majority of the vector DNA was found in an episomal (as concatemers) form. A near-random distribution of IS was detected across the genome. The observed integration profile was not associated with insertion close to or within genes implicated in malignant transformation.

4.3.4. Tumorigenicity

The applicant did not conduct dedicated carcinogenicity/tumorigenicity studies, nor did they submit any data or summary. Instead, carcinogenic/tumorigenic risk was informed by the evaluation of vector DNA integration into liver genomic DNA (refer to Section 4.3.3. for details).

In the GLP toxicity studies, histopathological evaluation of the liver tissues from mice and NHPs 6 months post-AAV administration showed no abnormalities, such as hypertrophy or hyperplasia, that could indicate potential oncogenicity.

4.3.4.1. Tumorigenicity Summary:

- The tumorigenicity or carcinogenicity studies of etranacogene dezaparvovec or AMT-060 were not conducted. No abnormalities that could indicate potential oncogenicity were identified in the histopathological evaluation of the liver tissues from mice and NHPs in the GLP toxicity studies.

4.3.5. Developmental and Reproductive Toxicology (DART) Study

No conventional DART or JAS studies were conducted with etranacogene dezaparvovec.

Biodistribution studies with AMT-060 in male mice and NHPs indicated the presence of vector DNA in reproductive organs. A subsequent germline transmission study was therefore performed, but the applicant did not explain the reason why they did not conduct the standard DART studies.

As hemophilia B is almost exclusively limited to male patients, and the application is only for adult patients, dedicated EFD and PPND studies or JAS studies are deemed not warranted currently. Anyhow, although hemophilia is rarely seen in women, it is recommended to reflect the unknown risk regarding females receiving etranacogene dezaparvovec in the labeling.

Studies/Species	Dose/Route	Significant drug-related events
Paternal germline transmission (to untreated females and their offspring)		
WT mouse (15♂30♀) Initial age: 6~9 wks [NR-060-14-001] GLP: Yes TF: CRL 1st dosing: N/A	AMT-060 0, $2.3x10^{14}$ gc/kg Single dose i.v. ♂: -d6 prior mating ♀: untreated Vehicle: PBS + 5% sucrose	Parameters: CS, BW, FC, Macro, OW, Micro, mating performance, fertility indices, and pregnancy performance, external fetal abnormalities, fetal weights; vector DNA in male and female reproductive organs and fetuses (Section 4.2.5); ▪ No TA-related effects ▪ NOAEL=$2.3x10^{14}$ gc/kg

CS: Clinical Sign; BW: Body Weight; FC: Food Consumption; OP: Ophthalmology; ECG: Electrocardiography; Hema: Hematology; CC: Clinical Chemistry Urin: Urinalysis; OW: Organ Weight; Macro: Macroscopy; Micro: Microscopy; TK: Toxicokinetics; OT: Others.
+: minimal; ++: mild or slight; +++: moderate; ++++: marked or severe; +++++: very severe or massive

4.3.5.1. Developmental and Reproductive Toxicology (DART) Summary:

- In a germline transmission study in AMT-060 treated male mice mated with untreated female mice, no adverse effects on reproductive performance were observed.

4.3.6. Immunogenicity and Immunotoxicity

No dedicated studies were conducted. The immune response against the AAV5 capsid was assessed in the monkey GLP toxicity studies of AMT-060 or etranacogene dezaparvovec.

A clarification/justification regarding the assessment of immunogenicity was provided (see Applicant's submission; Non-Clinical Overview) and summarized below.

- Antibody responses against the hFIX protein were not evaluated in mice treated with AMT-060 or etranacogene dezaparvovec considering the stable hFIX protein levels. As per the published studies, a lack of humoral immune response against hFIX protein in hemophilic and WT mice was reported. The immune tolerance for hFIX in these mouse models may have contributed to the high FIX protein levels achieved in these mice.
- In individual NHPs treated with AMT-060 or etranacogene dezaparvovec, the hFIX protein levels declined with time, which was attributed to an immune response against the circulating hFIX protein. Cross-species immune responses against hFIX protein have been reported to occur in NHPs in spite of the high homology of the FIX protein in rhesus and cynomolgus macaques as compared to the hFIX protein (97%).
- Antibodies directed against hFIX were detected in 8 of the 27 cynomolgus macaques treated with AMT-060 or etranacogene dezaparvovec. The antibody responses were associated with a trend of declining FIX levels towards the end of the 6-month observation period. The macaque with the highest level of antibodies showed a complete absence of hFIX protein in plasma by week 8 after infusion with etranacogene dezaparvovec at $2.5x10^{13}$ gc/kg. Overall, across both studies there was no clear evidence for a relationship between vector dose and extent of antibody formation in the monkey studies.

4.3.7. Other Toxicity Studies

N/A

4.3.8. Studies on Metabolites

N/A

4.3.9. Studies on Impurities

N/A

4.3.10. Summary of Systemic Exposure Data from Pivotal Toxicology Studies and from the Maximum Recommended Human Dose (MRHD)

Exposure data of human studies			
MRHD (gc/kg)		**Systemic exposure for the MRHD based on AUC_{0-24}** (ng*hr/mL)	
$2x10^{13}$		N/A	
Exposure data of pivotal toxicology studies			
Study/species/ duration	**NOAEL** (gc/kg)	**Systemic exposure for the NOAEL based on AUC** (ng*hr/mL)	**Safety margin**[#] (Multiple of NOAEL/MCHD)
Single-dose toxicity studies			
Ob for 6-months/ ♂mouse/ IV	$2.3x10^{14}$	N/A	10
Ob for 13-week/♂mouse/ IV	$5x10^{13}$	N/A	2.5
Ob for 6 months/ ♂monkey/ IV	$9x10^{13}$	N/A	5
Ob for 26-week/♂monkey/ IV	$9x10^{13}$	N/A	5
Reproductive toxicity studies			
Paternal germline transmission, ♂mouse	$2.3x10^{14}$	N/A	10
Carcinogenicity studies: N/A			

ND: not determined; N/A: not available or not applicable; [#]: safety margin was calculated on the basis of body weight.

4.4. External Communication (deficiency/inquiry and supplement, expert consultation…etc.)

The applicant has revised the proposed labeling per reviewer's request. The revision is acceptable.

4.5. Conclusion and Recommendation

Etranacogene dezaparovovec is a recombinant AAV5 vector containing a transgene encoding the gain-of-function Padua-variant (R338L) of human FIX (hFIXco-Padua) under the control of LP1 promoter. The non-clinical studies were first conducted with AMT-060 encoding wild-type (WT) human FIX, and then with etranacogene dezaparvovec.

Long-term persistence of vector DNA in the liver and sustained plasma hFIX protein expression up to 18 months post-dosing were demonstrated in mice dosing AMT-060 at $2.3x10^{14}$ gc/kg at different ages. In a mouse model of hemophilia B, IV administration of AMT-060 at doses of $5 x10^{11}$ to $2.3x10^{14}$ gc/kg resulted in dose-dependent increases in vector transduction in the liver, plasma hFIX protein levels and plasma hFIX activity.

In WT mice and non-human primates (NHPs), single-dose IV administration of AMT-060 or etranacogene dezaparvovec at doses of $5 x10^{11}$ to $5 x10^{13}$ gc/kg and $5x10^{12}$ to $9x10^{13}$ gc/kg, respectively, resulted in a dose-dependent increase in plasma hFIX protein levels and a dose-response relationship in FIX chromogenic and clotting activities. At equal doses, etranacogene dezaparvovec-

treated animals showed higher hFIX chromogenic and clotting activity compared to animals receiving AMT-060. In NHPs, the decreased hFIX protein levels and associated FIX activity observed in individual animals were attributed to the developed anti-hFIX Ab.

In mice and NHPs, IV administration of etranacogene dezaparvovec and AMT-060 resulted in a similar profile of vector DNA in the plasma, liver, and extrahepatic tissues. At 13 weeks post-dosing in mice receiving $5x10^{13}$ gc/kg of etranacogene dezaparvovec, plasma vector DNA levels peaked on day 1 and gradually decreased by the end of the study. Vector DNA was detected in all tissues examined, with the highest level in the liver, followed by adrenal glands, and lowest in bone marrow.

In NHPs, the kinetics of vector DNA in the serum were similar to those observed in mice. The AMT-060 vector DNA levels in saliva and urine were multiple logs lower than in serum and followed a similar clearance profile for serum. Measurable, trace, or low amounts of vector DNA could be detected in semen collected from animals receiving $\geq 2.5x10^{13}$ gc/kg of AMT-060. At 26 weeks post-dosing in NHPs receiving $5x10^{12}$ to $9x10^{13}$ gc/kg of etranacogene dezaparvovec, vector DNA was detected in all tissues (including testis, epididymis and seminal vesicles), in a dose-dependent manner, with highest level in the liver, followed by adrenal glands and spinal cord. In the rest of the extrahepatic tissues, vector DNA levels were 1-2 log lower than in liver. Quantifiable hFIX mRNA was primarily detected in the liver and also detected at levels close to the LLOQ in the adrenals, spleen, kidney, spinal cord, and heart in some animals treated with higher doses. [redacted]

GLP single-dose IV toxicity studies of AMT-060 or etranacogene dezaparvovec at doses up to 10- and 5-fold the recommended human dose of $2x10^{13}$ gc/kg in mice and monkeys, respectively, with an observation period of up to 6 months, were generally well-tolerated. No AMT-060- or etranacogene dezaparvovec-related adverse effects were observed except for non-adverse effects such as minimal pulmonary thrombi observed in two mice treated at the highest dose, and a transient mild elevation of liver enzymes. No effects were observed on safety pharmacology endpoints, including clinical observations and ECG results. The anti-drug antibodies against the AAV5 vector could be observed in NHPs at the end of the 6-month observation period.

In an integration site analysis study conducted with liver tissues collected 6 months after infusion, from mice and NHPs receiving AMT-060, a measurable level of integration of vector DNA in both species was found. The majority of the vector DNA was found in an episomal (as concatemers) form. A near-random distribution of IS was detected across the genome. The observed integration profile was not associated with insertion close to or within genes implicated in malignant transformation.

The tumorigenicity or carcinogenicity studies of etranacogene dezaparvovec or AMT-060 were not conducted. No abnormalities that could indicate potential carcinogenicity were identified in the histopathological evaluation of the liver tissues from mice and NHPs in the GLP toxicity studies.

In a paternal germline transmission study in AMT-060-treated male mice mated with untreated female mice, vector DNA was detected in the male reproductive tissues in the AMT-060-treated male

mice, but not detected in the fetus and female reproductive tissues of the untreated female mice. No adverse effects on reproductive performance were observed as well.

Overall, the non-clinical program done to support the NDA for **Hemgenix®** is considered acceptable. In general, the non-clinical information in the proposed labeling is adequate. From the non-clinical Pharm/Tox perspective, this NDA is recommended to be approved.

5. Pharmacokinetics / Pharmacodynamics Evaluation

5.1. Introduction

This application of Hemgenix filed by 傑特貝林有限公司. Hemgenix is a **somatic gene therapy medicinal product** that aims to deliver Factor IX (FIX) to the liver of patients suffering from hemophilia B. The therapy employs a non-replicating, recombinant adeno-associated viral vector serotype 5 **(AAV5)** containing a codon-optimized coding DNA sequence for the human coagulation Factor IX variant **R338L (hFIXco-Padua)** under the control of a liver-specific promoter (LP1). Vector DNA is maintained almost exclusively in episomal form.

The active ingredient of Hemgenix is **etranacogene dezaparvovec** (also called **AMT-061 or AAV5-hFIXco-Padua**). Hemgenix ® is indicated for treatment of adults with Haemophilia B (congenital Factor IX deficiency) and with a preexisting neutralising AAV5 anti body titre below 1:900 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.

Hemgenix have gotten approval in US FDA and EMA. The approval posology and indication were shown as follows:

	US FDA	**EMA**
License No.	BLA 125772	EMEA/H/C/004827
Product name	HEMGENIX suspension, for intravenous infusion	Hemgenix 1 x 10^{13} genome copies/mL concentrate for solution for infusion
Proper Name	etranacogene dezaparvovec-drlb	etranacogene dezaparvovec
Manufacturer	[redacted]	[redacted]
Indication	HEMGENIX is an adeno-associated virus vector-based gene therapy indicated for the treatment of adults with Hemophilia B (congenital Factor IX deficiency) who: • Currently use Factor IX prophylaxis therapy, or • Have current or historical life-threatening hemorrhage, or • Have repeated, serious spontaneous bleeding episodes.	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.
Pososlogy	***For single-use intravenous infusion only.*** • Perform baseline testing to select patients, including testing for Factor IX inhibitor presence and liver health tests. • The recommended dose of HEMGENIX is 2 x 10^{13} genome copies (gc) per kg of body weight. • Administer HEMGENIX as an intravenous infusion after dilution with 0.9% normal saline at a constant infusion rate of 500 ml/hour (8 mL/min).	The recommended dose of Hemgenix is a single dose of 2 x 10^{13} gc/kg body weight corresponding to 2 mL/kg body weight, administered as an intravenous infusion after dilution with sodium chloride 9 mg/mL (0.9%) solution for injection. ***Hemgenix can be administered only once.***

The quantitative composition of the formulated DP and the respective function and quality standard of the various components is presented in Table X. **Each mL** of etranacogene dezaparvovec contains a nominal concentration of **1 × 10^{13} genome copies (gc)** in **PBS pH 7.1** with [redacted] **sucrose** and [redacted] **PS-20**.

[redacted]

Points of review:

- Shedding of vector DNA (clearance of vector DNA) in body fluids
- The kinetics of FIX activity and FIX protein following etranacogene dezaparvovec administration
- The impact of immunogenicity on FIX activity
- Possible drug-drug interaction
- Dosage recommendation for special populations

Table 1. Summary of Hemgenix® pharmacokinetics.

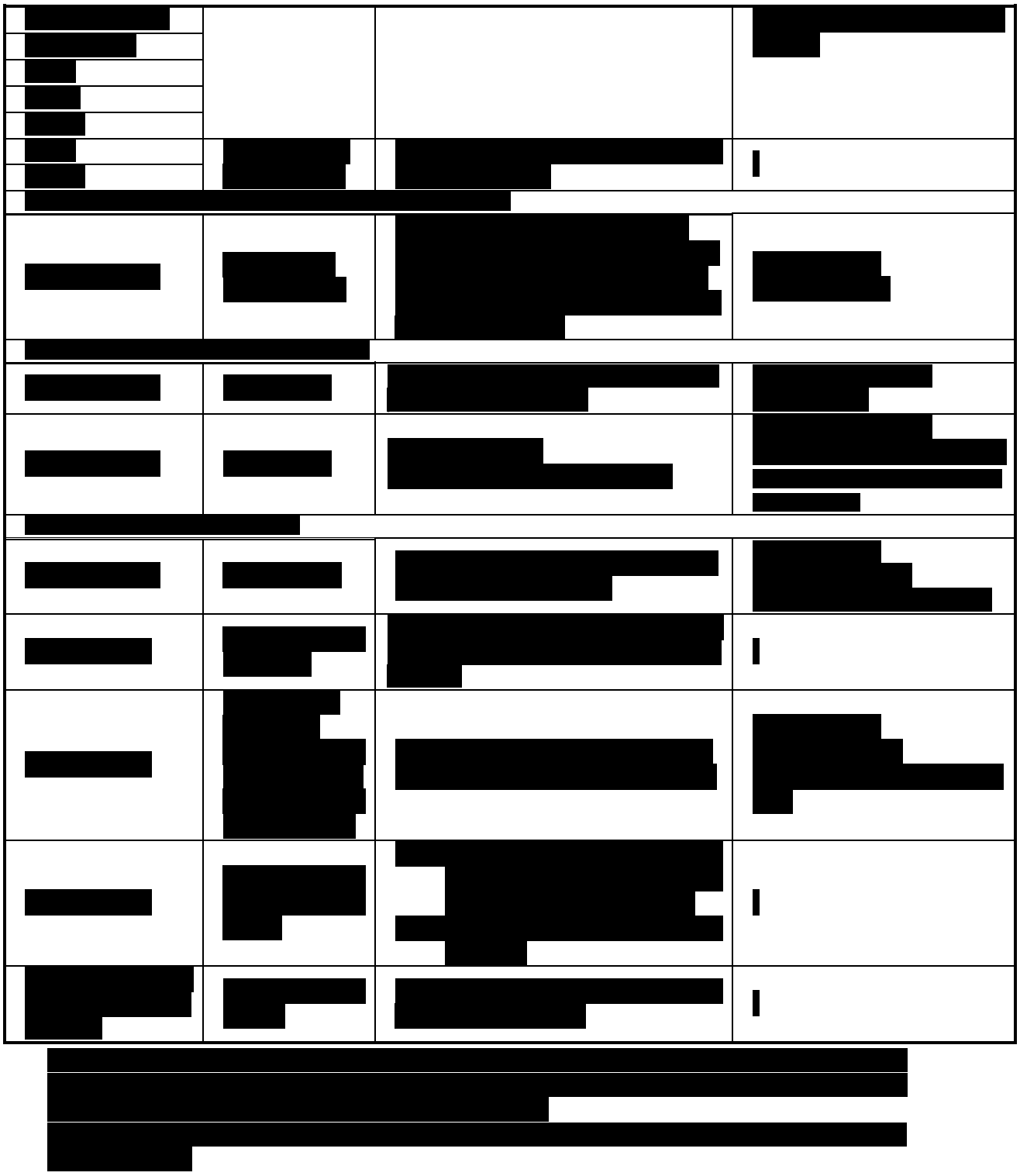
PK/PD Assessment	PK Parameter/Finding/Comment
Recommended dosage regimen	A single dose of 2 × 10^{13} gc per kilogram (kg) of body weight (bw) or 2.0 mL/kg bw, administered as an intravenous (IV) infusion after dilution with 0.9% sodium 90 chloride solution (normal saline).

Absorption	Absolute bioavailability: 100% due to IV infusion Intra-subject variation: N/A Inter-subject variation: 46.6% - 58% Food effect: N/A
Disposition Kinetics	unknown
Single Dose (Study CT-AMT-061-02)	The FIX activity showed sustained clinically relevant levels (mean FIX activity > 36%) from Month 6 to Month 36 postdose, following a single dose of 2×10^{13} gc/kg (Table 5 and updated Table)
Multiple Dose	Hemgenix is a gene therapy drug and is for single use only.
Distribution Metabolism Excretion	Hemgenix is a gene therapy drug, traditional PK study is not suitable to evaluate.
Drug Interactions	No drug-drug interaction studies have been performed.
Renal Impairment	Though Hemgenix has not been studied in patients with renal impairment, no dose adjustments were recommended. The safety and efficacy in patients with severe renal impairment and endstage renal disease have not been studied
Hepatic Impairment	Hemgenix has not been studied in patients with hepatic impairment. No clinically meaningful differences in FIX activity (< 30% relative difference between < S2 and ≥ S2 groups) were observed. No dose adjustments are recommended in patients with hepatic disorders. And the safety and efficacy of etranacogene dezaparvovec in patients with severe hepatic impairment have not been studied.
Effect of age on PK	Elderly: There was a trend of higher FIX activity with increase in age. No dose adjustments are recommended Pediatrics: have not been evaluated in subjects of < 18 years of age.
Effect of gender on PK	N/A. Most hemophilia B patients are male, since males have only one X chromosome. Once the factor IX gene is missing on a boy's X chromosome, he will have Hemophilia B.
PK/PD	Hemgenix can deliver hFIX39-R338L to liver, and induce body to produce FIX protein with higher FIX activity.
Population PK/PD	N/A

5.2. Formulation, dosage and drug administration

Formulation	Concentrate for solution for infusion
Dosage regimen	**For single-dose intravenous infusion only.** ● The dose of etranacogene dezaparvovec is **a single dose of 2×10^{13} gc per kilogram (kg) of body weight (bw) or 2.0 mL/kg bw**, administered as an intravenous infusion after dilution with 0.9% sodium 90 chloride solution (normal saline). ● **The dose should be calculated as follows:** Etranacogene dezaparvovec dose (in mL) = patient body weight (in kilogram) × 2 **Etranacogene dezaparvovec can be administered only once.**
Route of administration	Intravenous (IV) infusion

5.3. Bioanalytical methods



No significant different for FIX activity values between central laboratory and local laboratories.

The revised Chinese labeling (revised date: 2024.6.28) contained the following information which is stated in both US FDA package insert and EMA SPC.

There was no interference tests about whether the presence of the FIX-R338L protein from Hemgenix will affect the FIX activity test results of other Factor IX replacement products.

5.4. PK/PD

5.4.1. Pharmacokinetics

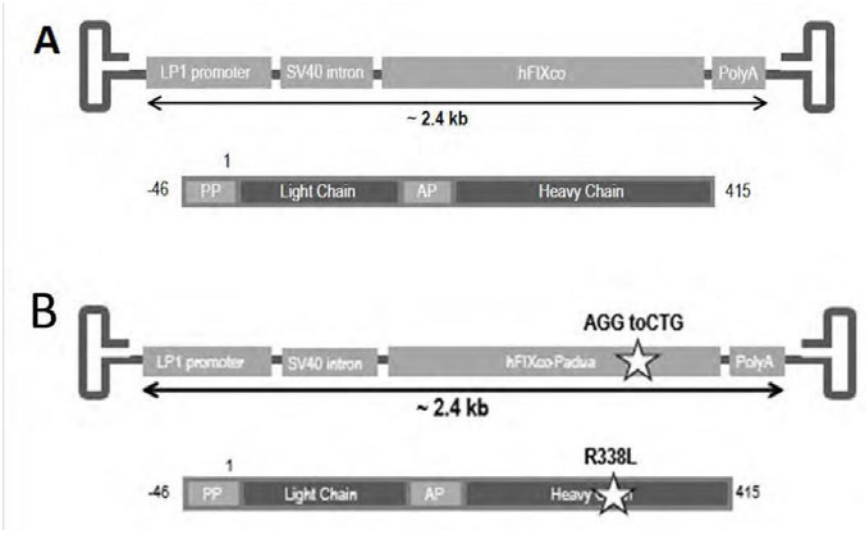
[redacted]

Etranacogene dezaparvovec (**AMT-061**) has a predecessor, **AMT-060**, a modified derivative of AAV5-hFIXco containing the codon-optimized coding DNA sequence **wild-type human FIX (hFIXco)**.

Etranacogene dezaparvovec and AMT-060 differ by a 2-nucleotide change in the coding sequence of the expression cassette, which results in a single amino acid change in the therapeutic transgene product, with ***arginine*** replaced by ***leucine*** at position 338 **(R338L)** of the mature FIX protein (**this can elevate 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. Following Figure (1) is a graphical presentation of the single stranded vector genome of AMT-060 (A) and etranacogene dezaparvovec (B) and the associated hFIX and hFIX-Padua proteins, respectively.

Figure 1. Structure of Vector Genomes and Corresponding Expressed Factor IX Protein, for (A) AMT-060 and (B) Factor IX-Padua for Etranacogene Dezaparvovec

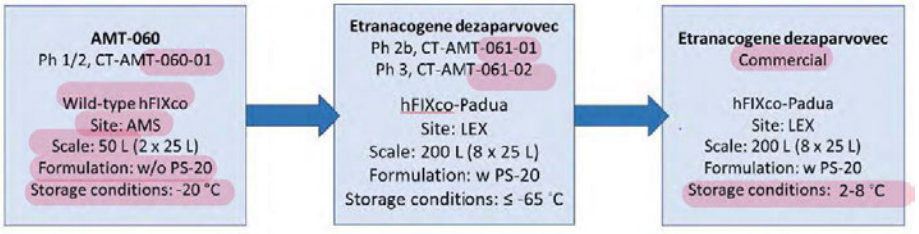


[redacted]; AP = activation peptide; [redacted] FIX = factor IX; hFIXco = codon-optimized human coagulation factor IX complimentary deoxyribonucleic acid; ITR = inverted terminal repeat; **LP-1 = liver-specific promoter**-1; PP = pre-pre-peptide.
A. Structure of **AMT-060 (AAV5-hFIXco)** vector genome and derived wild-type human FIX protein. The single stranded hFIXco expression cassette is flanked by 2 ITRs (hairpin structures) and consists of the LP-1 promoter, SV40 intron, hFIXco coding sequence, and SV40 poly(A), in that order. The vector genome is approximately 2.4kb in size. Below the vector genome, a schematic representation of the translated protein is provided (PP, AP).
B. Structure of **etranacogene dezaparvovec (AAV5-hFIXco-Padua)** vector genome and derived human FIX-Padua protein. The etranacogene dezaparvovec vector genome is identical to the AMT-060 vector genome except for a 2-nucleotide substitution (AGG to CTG as indicated). This substitution results in an arginine to leucine substitution in the translated protein, at position 338 (R338L) of the mature protein.

[redacted]

Concurrent with the formulation changes, etranacogene dezaparvovec was subsequently developed to enhance the FIX activity expressed per unit of dose administered, with modification of the transgene from hFIXco to the Padua variant of hFIXco (hFIX-Padua). Etranacogene dezaparvovec was studied in the phase 2b Study CT-AMT-061-01 and the pivotal phase 3 Study CT-AMT-061-02 (Figure 2). The proposed commercial formulation is identical to that used in Study CT-AMT-061-02, except for storage conditions (change from ≤ -65℃ to 2-8℃).

Figure 2. Overview of Major Changes During Drug Product Development



AMS = uniQure Amsterdam, The Netherlands manufacturing facility; LEX = uniQure Lexington, Massachusetts, United States manufacturing facility; PS-20 = polysorbate 20; w = with; w/o = without.
(Source: m2.7.1 Figure 2)

There were three clinical trials to investigate the kinetics of FIX activity and FIX protein following AMT-060 (AAV5-hFIXco) and AMT-061 (etranacogene dezaparvovec) administration. The study design was shown in following Table 2.

FIX activity was measured by both one-stage (aPTT-based) FIX assay and chromogenic FIX assay. Only values of **FIX activity or protein** that were measured **more than 10 days** after most recent FIX-replacement therapy administration were

included, and are referred to as **FIX-replacement free or uncontaminated values**. Sampling for **vector DNA** was continued for the individual subject and for a specific matrix, only **until 3 consecutive negative samples** were detected for the subject and for that particular type of matrix.

Table 2. The Study Design of Clinical Trials

Study Number	Study design	Population	Treatments (Number of sisbjects)	Analysis items (Analyte/Matrix)
CT-AMT-060-01	A phase 1/2, open-label, uncontrolled, single-dose, dose-ascending, multicenter study (with 5 years of follow-up)	Adult (male) subjects with moderately severe or severe hemophilia B	**Cohort 1**: 5 subjects received **AMT-060 5 × 10^{12} gc/kg** **Cohort 2**: 5 subjects received **AMT-060 2 × 10^{13} gc/kg**	● **FIX activity and FIX protein**: **Plasma** ● **Vector DNA: Blood, urine, saliva, nasal secretions; Semen, feces** ● NAbs to AAV5 capsid and total antibodies (IgG and IgM) to AAV5**:** Serum ● Anti-FIX antibodies: Serum ● FIX inhibitors: Serum ● AAV5 Capsid-specific T-cell response: Peripheral blood mononuclear cell (PBMC)
CT-AMT-061-01	A phase 2b, open-label, single-dose, single-arm, multicenter study		3 subjects received Etranacogene dezaparvovec **2 × 10^{13} gc/kg**	● FIX activity and FIX protein: Plasma ● **Vector DNA**: **Blood; Semen** ● NAbs to AAV5 capsid and total antibodies (IgG and IgM) to AAV5: Serum ● Anti-FIX antibodies: Serum ● FIX inhibitors: Serum ● AAV5 Capsid-specific T-cell response:PBMC
CT-AMT-061-02 [redacted]	phase 3, open-label, single-dose, **single-arm,** multicenter, multinational study		53 subjects received Etranacogene dezaparvovec **2 × 10^{13} gc/kg** *1 subject received approximately **10% of the 2 × 10^{13} gc/kg dose***	● FIX activity and FIX protein: Plasma ● **Vector DNA**: **Blood; Semen** ● NAbs to AAV5 capsid and total antibodies (IgG and IgM) to AAV5: Serum ● Anti-FIX antibodies: Serum ● AAV5 Capsid-specific T-cell response: PBMC

AAV = adeno-associated virus; AAV5-hFIX = recombinant serotype 5 AAV vector containing a codon-optimized human coagulation FIX cDNA; AAV5 hFIXco Padua = Recombinant adeno-associated viral vector containing a codon-optimized Padua variant of human coagulation factor IX cDNA; cDNA = complementary deoxyribonucleic acid; FIX = factor IX; gc = genome copies; IgG = immunoglobulin G; IgM = immunoglobulin M; NAbs = neutralizing antibodies.

- Known **severe** FIX deficiency with plasma FIX activity level <1% and a severe bleeding phenotype defined by 1 of the following:
  -At the time of screening, on prophylactic FIX replacement therapy for a history of bleeding.
  - At the time of screening, on on-demand FIX replacement therapy with a current or past history of frequent bleeding defined as 4 or more bleeding episodes in the last 12 months or chronic hemophilic arthropathy (pain, joint destruction, and loss of range of motion) in 1 or more joints.
- Known **moderately severe** FIX deficiency with plasma FIX activity level between ≥1% and ≤2% and a severe bleeding phenotype defined by 1 of the following:
  - At the time of screening, on prophylactic FIX replacement therapy for a history of bleeding.
  - At the time of screening, on on-demand FIX replacement therapy with a current or past history of frequent bleeding defined as 4 or more bleeding episodes in the last 12 months or chronic hemophilic arthropathy (pain, joint destruction, and loss of range of motion) in 1 or more joints.

(Source: m 5.2/ Table 1; m2.7.2 Table 1, Table 2, 5 and 7)

5.4.1.1. Absorption

5.4.1.1.1. Healthy & patient subjects

Etranacogene dezaparvovec is administered IV and is a gene therapy medicine product, no traditional absorption data can be acquired. The kinetics of FIX activity and FIX protein following etranacogene dezaparvovec or AMT-060 administration were presented as follows.

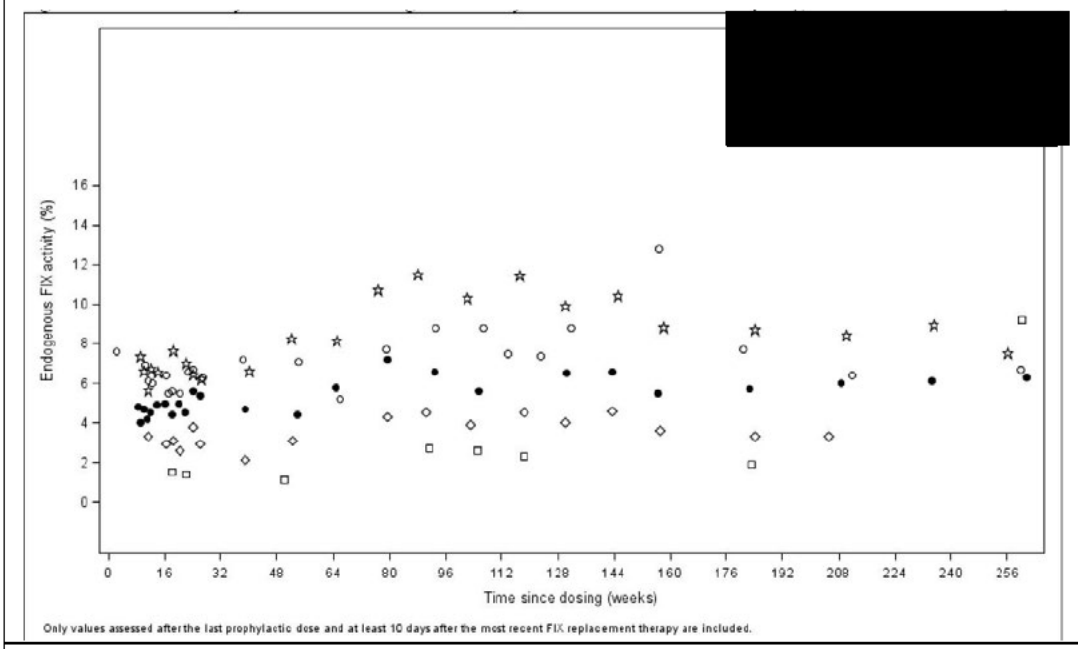
[redacted]

The FIX activity over time after a single-dose of AMT-060 were shown in Figure 3–(A) and Figure 3–(B). The mean endogenous FIX activity levels for each subjects in Cohort 1 (low dose) and Cohort 2 (high dose) ranged from 2.8% to 8.2% of normal, and 4.0% to 10.7% of normal, respectively, based upon the one-stage (aPTT-based) FIX assay. The FIX activity levels were stable during the post-tapering period. Three (3) of 5 subjects in Cohort 1 converted from a severe (2 subjects) or moderately-severe (1 subject) to a mild (i.e. >5%) FIX deficiency. And, four of the 5 subjects in Cohort 2 achieved a mean FIX activity of >5% (from severe to mild).

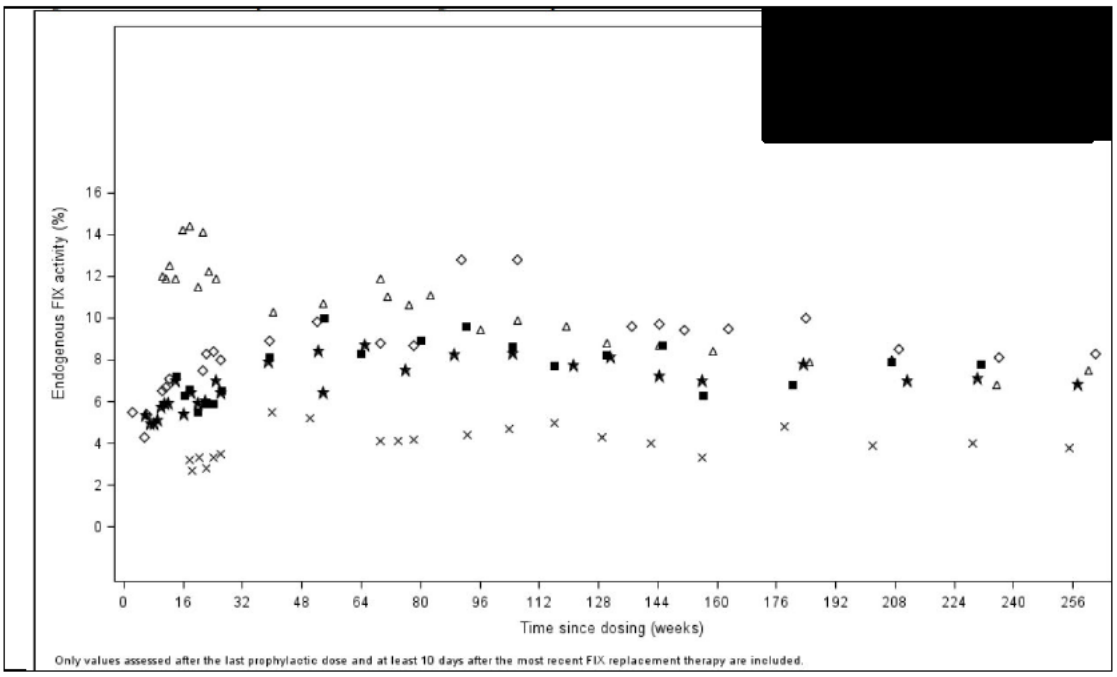
The Uncontaminated FIX activity (%) and FIX Protein Concentration (%) at selected visits were presented in Table 3. The correlation between FIX replacement-free FIX activity and FIX protein levels is presented in Figure 4.

Figure 3. Uncontaminated Endogenous FIX Activity Over Time for (A)Cohort 1 and (B) Cohort 2 in Study CT-AMT-060-01 – Individual Values

(A) Cohort 1



(B) Cohort 2



(Source: m2.7.2 Figure 1; CSR CT-AMT-060-01 Figure 14.2.21 and Figure 14.2.22)

Table 3.Uncontaminated FIX Activity and FIX Protein in the Post-treatment Period at Selected Visits (FAS= Full analysis set)

Visit[a] / Statistic	Cohort 1			Cohort 2		
	FIX Activity (%) by One-Stage (aPTT-based) FIX Assay[b] (N = 5)	FIX Activity (%) by Chromogenic Assay (N = 5)	FIX Protein (%) (N = 5)	FIX Activity (%) by One-Stage (aPTT-based) FIX Assay[b] (N = 5)	FIX Activity (%) by Chromogenic Assay (N = 5)	FIX Protein (%) (N = 5)
Baseline, n[c]	-	-	-	1	1	1
Mean (SD)				0.30 (-)	0.40 (-)	0.62 (-)
Week 6, n	-	-	-	2	2	2
Mean (SD)				5.35 (0.07)	3.25 (0.07)	4.58 (0.94)
Month 6 (Week 26), n	4	4	4	5	5	5
Mean (SD)	5.20 (1.59)	3.10 (1.37)	20.86 (28.85)	7.26 (3.06)	5.06 (2.46)	27.24 (43.53)
Month 12 (Week 52), n	3	3	3	5	5	5
Mean (SD)	5.23 (2.65)	3.10 (1.50)	6.40 (4.97)	8.82 (2.19)	5.34 (2.15)	25.41 (40.25)
Month 18 (Week 78), n	4	4	4	5	5	5
Mean (SD)	7.48 (2.62)	4.15 (1.69)	22.93 (30.62)	7.98 (2.38)	5.56 (2.06)	21.64 (30.53)
Month 24 (Week 104), n	5	5	5	5	5	5
Mean (SD)	6.24 (3.25)	3.60 (2.14)	18.32 (28.02)	8.86 (2.93)	5.08 (2.08)	28.71 (46.05)
Month 36 (Week 156), n	4	3	4	5	5	5
Mean (SD)	7.68 (4.04)	5.47 (2.82)	28.34 (43.14)	6.90 (2.36)	5.88 (2.23)	26.74 (43.80)
Month 48 (Week 208), n	3	3	3	5	5	5
Mean (SD)	5.23 (1.69)	3.40 (1.40)	30.96 (41.23)	7.06 (1.85)	3.94 (1.38)	28.99 (48.70)
Month 60 (Week 260), n	4	4	4	4	5	4
Mean (SD)	7.43 (1.28)	4.58 (2.88)	24.02 (29.02)	6.60 (1.96)	4.74 (1.43)	26.21 (40.13)

AAV5-hFIXco = recombinant AAV vector containing a codon-optimized human coagulation FIX; aPTT = activated partial thromboplastin time; cDNA = complementary deoxyribonucleic acid CSR = Clinical Study Report; FAS = Full Analysis Set; FIX = factor IX; gc = genome copies; SD = standard deviation.

a The CSR source tables for this study list data by visit number. For consistency, these have been described by week number in this document as follows: **Week 6 = Visit 8; Week 26 = Visit 21; Week 52 = Visit 23; Month 18 (Week 78) = Visit 25; Month 24 (Week 104) = Visit 27; Month 36 (Week 156) = Visit 31; Month 48 (Week 208) = Visit 33; Month 60 (Week 260) = Visit 35.** Where uncontaminated data was not available for specific visits, cells have been left blank.

b Only assessments performed more than 10 days after most recent FIX-replacement therapy administration included.

c Baseline was defined as the assessment done at the predose visit on dose date if non-missing and, if missing, as the last assessment done before dosing. Baseline values are those taken at Visit 1.

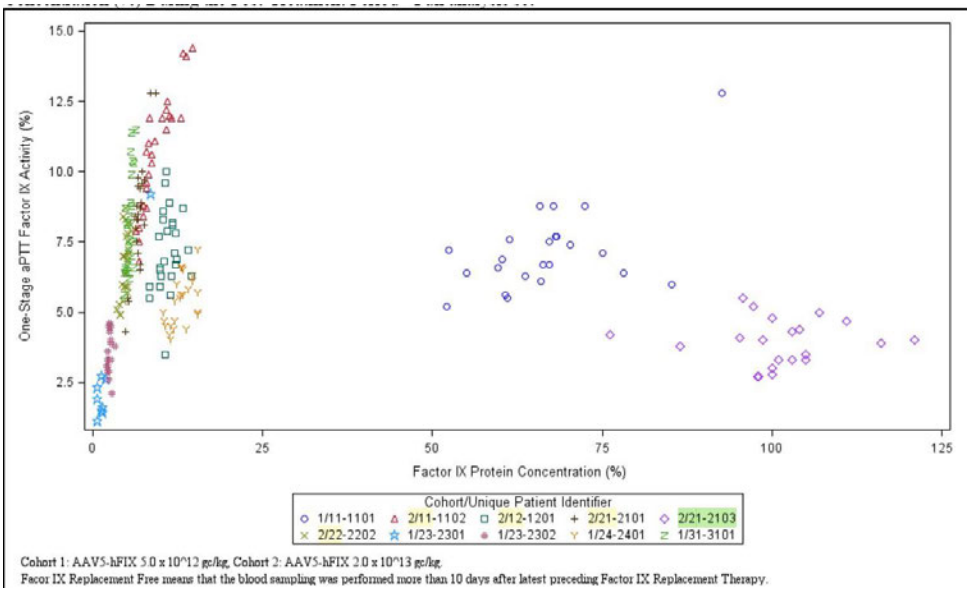
1. Cohort 1: AAV5-hFIXco (AMT-060) 5×10^{12} gc/kg, Cohort 2: AAV5-hFIXco (AMT-060) 2×10^{13} gc/kg.

2. Only assessments performed more than 10 days after most recent FIX-replacement therapy administration were included.

Source: CT-AMT-060-01 5-year CSR, Table 14.2.2, Table 14.2.4, Table 14.2.31.

(Source: m2.7.2 Table 3; CSR CT-AMT-060-01/Table 19; CT-AMT-060-01 5-year CSR, Table 14.2.2, Table 14.2.4, Table 14.2.31)

Figure 4. Scatterplot of Factor IX Replacement Free One-Stage aPTT Assay for Factor IX Activity (%) Versus Factor IX Protein Concentration (%) During the Post-Treatment Period – FAS



(Source: CSR CT-AMT-060-01/ Figure 14.2.25)

[redacted]

This study was to confirm that a single dose of 2×10^{13} gc/kg etranacogene dezaparvovec resulted in FIX activity levels of ≥ 5% at 6 weeks after dosing in adult male patients with moderately severe or severe congenital hemophilia B. After dosing, subjects are being followed for a total of 5 years (60 months) for evaluation of efficacy parameters and safety.

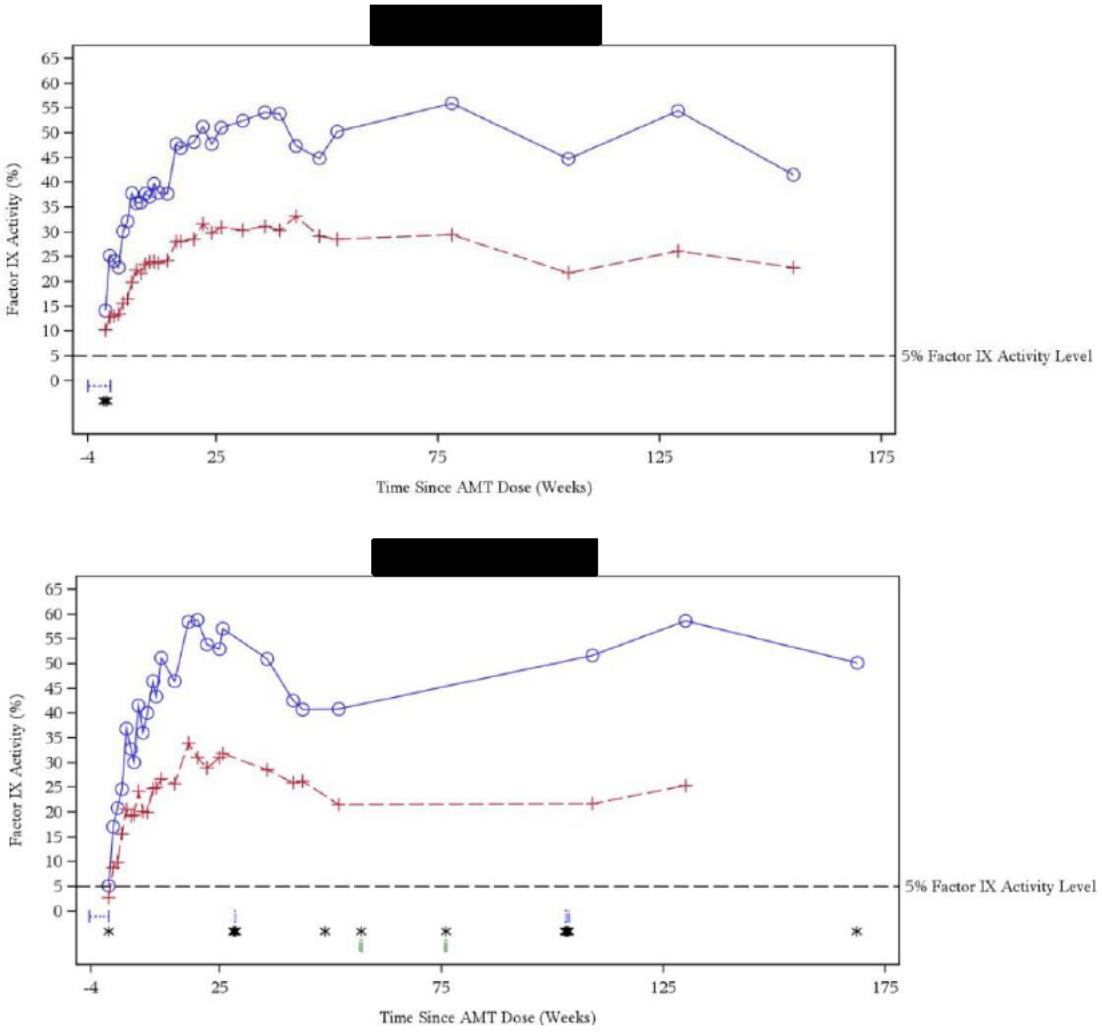
All three (3) subjects experienced clinically relevant increases in FIX activity (Figure 5). The mean FIX activity level at 6 weeks, 52 weeks, 24 months and 2.5 years (30 months) were 30.6%, 40.8%, 44.2% and 50.0%, respectively, based on one-stage (aPTT-based) assay (Table 4). At 3 years (36 months), the FIX activity values from 2 subjects were 32.3% and 41.5%, respectively (*the FIX activity at Month 36 for one subject (10-11-102) was considered contaminated*).

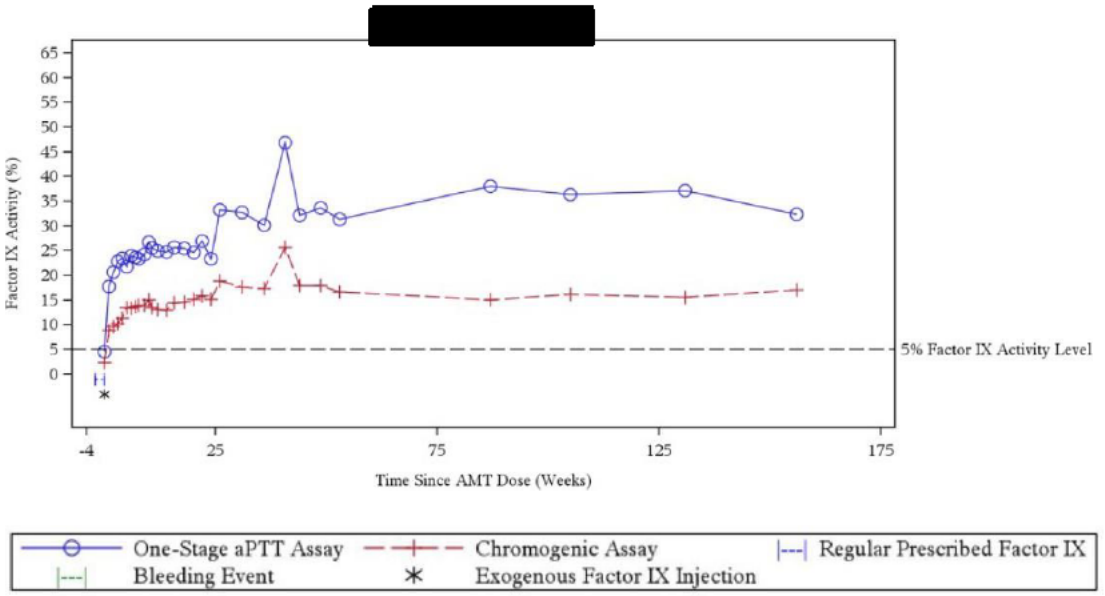
Exogenous FIX use after etranacogene dezaparvovec administration was also shown in Figure 5. Subject [redacted] did not require any FIX replacement therapy; Subject [redacted] received a single dose of FIX replacement therapy on Day 4 (4000 IU of BeneFIX®), and not due to a bleed. Besides, Subject [redacted] required replacement therapy per protocol for invasive procedures and not lack of efficacy.

The mean uncontaminated FIX protein concentration ranged from 3.595% to 4.237% between Week 3 and Month 36 (3 years) [The uncontaminated FIX protein concentration for individual subject was: [redacted]

The mean ratio of FIX activity to FIX protein was 5.085 at Week 3, gradually increasing to 7.167 at Week 6, and 8.267 at Week 52 (Table 4). At Month 18, Month 24, Month 30, and Month 36, the mean ratios were 12.785, 10.147, 11.874, and 10.131, respectively.

Figure 5. Factor IX Activity (%), Exogenous Factor IX Use, and Bleeding Episodes Over Time by Subject (All Subjects Treated)





A. "Contaminated" results were defined to be a value from a blood sample obtained within 5 half-lives of previous FIX therapy; exogenous FIX injections are shown in the graphs above.

B. Factor IX activity from the chromogenic assay was not available at Month 36 for Subject 10-11-102.

C. [redacted] Replacement therapy was provided for 5 days following elective hip surgery on Day 197 (Days 197 to 201; 1695 to 3710 IU BeneFIX) and for 7 days following elective hip arthroplasty on Day 720 (Days 720 to 726; 2000 to 6000 IU BeneFIX). This subject also received BeneFIX for on-demand use on Day 342 (1705 IU; single, self-administered infusion due to an unreported reason but not due to a reported bleed), Day 399 (1700 IU; for traumatic muscle bleed in lower leg), Day 533 (1700 IU; for spontaneous, mild muscle bleed in lower leg), and Day 1181 (1780 IU; prior to cortisone injection for ongoing TEAE of sciatica).

(Source: CSR CT-AMT-061-01/ Figure 2)

Table 4. Uncontaminated FIX Activity, FIX Protein, and the Ratio of FIX Activity to FIX Protein in the Post-treatment Period at Selected Visits (All Subjects Treated)

Visit Statistic	FIX Activity (%) by One-Stage (aPTT-based) Assay	FIX Activity (%) by Chromogenic Assay	FIX Activity Ratio of Chromogenic Assay to One-Stage (aPTT-based) Assay	FIX Protein (%)	Ratio of FIX Activity (One-Stage [aPTT-based] Assay) to FIX Protein
	(N = 3)	(N = 3)	(N = 3)	(N = 3)	(N = 3)
Baseline, n	1	1	1	N/A[a]	N/A[a]
Mean (SD)	5.10 (-)	2.70 (-)	0.5294 (-)	-	-
Week 3, n	3	3	3	3	3
Mean (SD)	23.40 (1.04)	13.07 (2.72)	0.5564 (0.0972)	4.703 (0.8517)	5.0848 (0.9234)
Week 6, n	3	3	3	3	3
Mean (SD)	30.57 (6.97)	17.50 (3.64)	0.5757 (0.0636)	4.310 (1.2372)	7.1666 (0.4899)
Month 3 (Week 12), n	3	3	3	3	3
Mean (SD)	37.97 (13.10)	21.13 (7.20)	0.5566 (0.0595)	3.790 (1.2344)	9.9852 (0.3054)
Month 6 (Week 26), n	3	3	3	3	3
Mean (SD)	47.07 (12.38)	27.17 (7.26)	0.5767 (0.0256)	4.190 (1.0776)	11.2307 (0.4143)
Month 9 (Week 40), n	3	3	3	3	3
Mean (SD)	47.70 (5.70)	27.27 (2.63)	0.5732 (0.0324)	4.270 (0.7762)	11.3260 (1.7269)
Month 12 (Week 52), n	3	3	3	3	3
Mean (SD)	40.77 (9.45)	22.20 (5.98)	0.5417 (0.0226)	4.897 (0.6741)	8.2672 (1.0014)
Month 18, n	2	2	2	2	2
Mean (SD)	46.95 (12.66)	22.20 (10.18)	0.4603 (0.0928)	3.725 (1.2940)	12.7853 (1.0435)
Month 24, n	3	3	3	3	3
Mean (SD)	44.20 (7.66)	19.83 (3.23)	0.4498 (0.0329)	4.353 (0.5859)	10.1470 (1.0249)
Month 30, n	3	3	3	3	3
Mean (SD)	50.03 (11.40)	22.30 (5.90)	0.4431 (0.0325)	4.237 (1.0758)	11.8743 (0.3916)
Month 36, n	2	2	2	2	2
Mean (SD)	36.90 (6.51)	19.90 (4.10)	0.5379 (0.0163)	3.650 (0.7212)	10.1313 (0.2197)

A. FIX activity or FIX protein concentration values that were measured more than 5 half-lives after most recent FIX-replacement administration are included here, and are referred to as uncontaminated values ("Uncontaminated" meant that the blood sampling did not occur within 5 half-lives of exogenous FIX use).

B. "Contaminated" results were defined to be a value from a blood sample obtained within 5 half-lives of previous FIX therapy. Both the data and time of exogenous FIX use (start) and the blood sampling were considering in determining contamination.

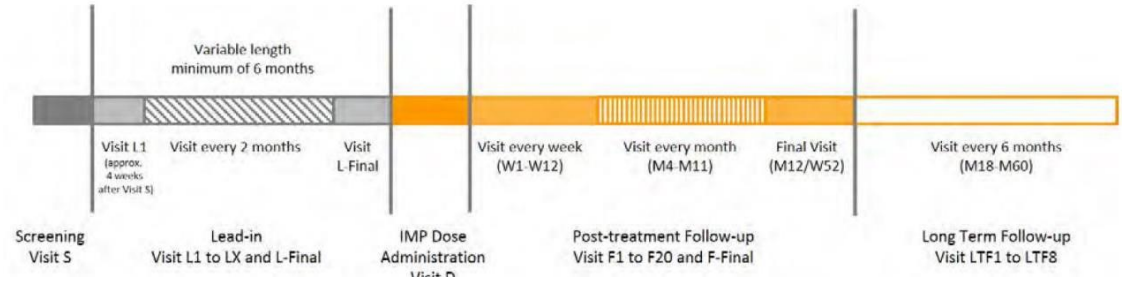
(Source: m2.7.2 Table 6; CSR CT-AMT-061-01/Table 9)

[redacted]

The pivotal study was to demonstrate the non-inferiority of etranacogene dezaparvovec (2×10^{13} gc/kg) during the 52 weeks following establishment of stable FIX expression (Months 6 to 18) post-treatment follow-up compared to standard of care (SOC) continuous routine FIX prophylaxis during the lead-in phase, as measured by the ABR. The study design diagram was as follows (Figure 6).

During the **lead-in phase** (which lasted for a minimum of 26 weeks (i.e., ≥6 months), subjects was allowed to use standard of care continuous routine FIX prophylaxis, and recorded their use of FIX replacement therapy and bleeding episodes. This data was used for comparison with the post-dose assessments of efficacy and safety. After the lead-in phase, subjects received a single-dose of etranacogene dezaparvovec at the dosing visit (Visit D) and were followed for 1 year (52 weeks) to evaluate efficacy and safety.

Figure 6. Study Design Diagram

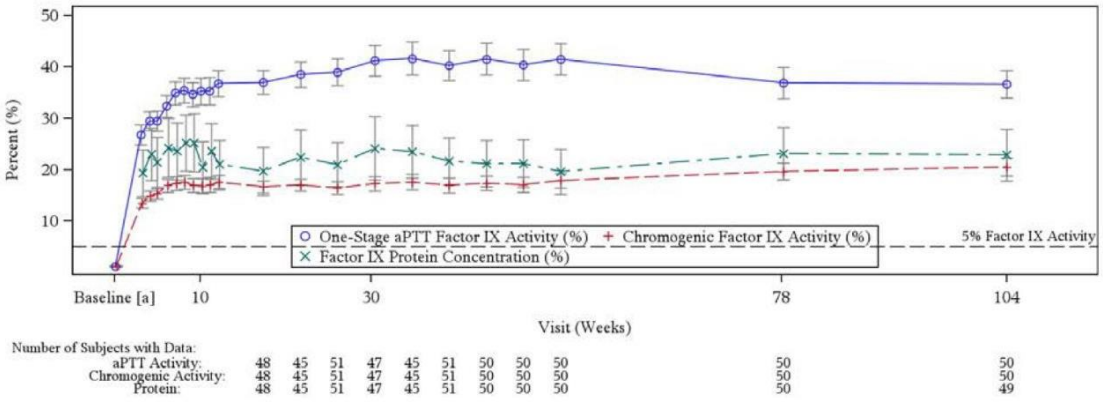


(Source: CSR CT-AMT-061-02/ Figure 1)

Clinically relevant and statistically significant increases in FIX activity were observed after administration of etranacogene dezaparvovec (Figure 7). The mean uncontaminated FIX activity level was increased from 1.19% (baseline) to 32.63% (at Week 6), and sustained 38.95% (at Month 6), 41.48% (at Month 12), 36.90% (at Month 18), and 36.66% (at Month 24) of normal, respectively, based on one-stage (aPTT-based) assay (Table 5). At Month 24, 50% of subjects achieved at least a 32.5% increase in FIX activity (Table 6). There were 5/50 (10%) subjects with FIX activity < 12% at Month 24 (refer to CT-AMT-061-02 2-year CSR, Table 2.2.8.6).

The mean FIX protein levels ranged from 19.35% to 25.25% during 24 months after treatment with etranacogene dezaparvovec (Table 5; refer to CSR Table 2.3.1). The mean ratio of FIX activity to FIX protein was 5.8673 at Week 3, gradually increasing to 6.6137 at Week 6, and 8.4819 at Week 52 (Table 5). At Month 18, Month 24, Month 30, and Month 36, the mean ratios were 7.1286, 6.9761, 9.5950, and 9.2329, respectively.

Figure 7. Mean (+ / - SE) of Uncontaminated Central Laboratory FIX Activity (%) by One-Stage (aPTT-based) and Chromogenic Assay and FIX Protein Concentration Over Time During the Post-Treatment Period



Subjects with zero uncontaminated central laboratory postdose values have their post-baseline activity values set equal to their baseline value. Baseline FIX activity was imputed based on subject's historical hemophilia B severity documented on the CRF. If the subject had documented severe FIX deficiency (plasma level < 1%), their baseline FIX activity was imputed as 1%. If the

subject has documented moderately severe FIX deficiency (plasma level ≥ 1% and ≤ 2%), their baseline activity level was imputed as 2%. The ten subject counts were for post-treatment Month 4, 5, 6, 7, 8, 9, 10, 11, 12, and 18, respectively. The SE was not provided at baseline.
(Source: m2.7.2 Figure 2)

Table 5. Uncontaminated FIX Activity, FIX Protein, and the Ratio of FIX Activity to FIX Protein in the Post-treatment Period at Selected Visits

Visit Statistic	FIX Activity (%) One-Stage (aPTT-based) Assay	FIX Activity (%) Chromogenic Assay	FIX Activity Ratio of Chromogenic Assay to One-Stage (aPTT-based) Assay	FIX Protein (%)	Ratio of FIX Activity (One-Stage [aPTT-based] Assay) to FIX Protein
Baseline, n	54	54	N/A	N/A[a]	N/A[a]
Mean (SD)	1.19 (0.39)	1.19 (0.39)			
Post-treatment Week 3, n	43	43	43	43	43
Mean (SD)	26.83 (12.71)	13.47 (6.11)	0.512 (0.071)	19.35 (30.01)	5.8673 (5.1952)
Post-treatment Week 6, n	47	47	47	47	47
Mean (SD)	32.36 (14.63)	16.91 (8.60)	0.513 (0.084)	24.23 (38.86)	6.6137 (4.3454)
Post-treatment Week 12, n	51	51	51	51	51
Mean (SD)	36.78 (18.17)	17.52 (9.90)	0.479 (0.103)	21.02 (33.02)	7.5997 (5.2440)
Post-treatment Month 6, n	51	51	51	51	51
Mean (SD)	38.95 (18.72)	16.45 (8.82)	0.414 (0.051)	20.96 (30.84)	7.5163 (4.8164)
Post-treatment Month 9, n	51	51	51	51	51
Mean (SD)	40.26 (20.91)	16.96 (10.29)	0.411 (0.048)	21.65 (32.69)	7.6662 (5.0663)
Post-treatment Month 12, n	50	50	50	50	50
Mean (SD)	41.48 (21.71)	17.86 (10.05)	0.422 (0.046)	19.64 (30.73)	8.4819 (5.4682)
Post-treatment Month 18, n	50	50	50	50	50
Mean (SD)	36.90 (21.40)	19.66 (11.72)	0.521 (0.060)	23.11 (36.30)	7.1286 (5.0667)
Post-treatment Month 24, n	50	50	50	49	49
Mean (SD)	36.66 (18.96)	20.50 (12.20)	0.547 (0.062)	22.87 (35.51)	6.9761 (4.8751)

a No true baseline was collected for FIX protein.
Uncontaminated central laboratory data was used; blood sampling 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.
Overall n was the number of observations across all post-treatment visits and subjects.
(Source: m2.7.2/Table 8; CSR CT-AMT-061-02/Table 17; CSR CT-AMT-061-02 24-month/Table 2.1.1.5 Sensitivity Analysis 4)

Table 6. Cumulative Proportion of Responders Based on **Change From Baseline** in Uncontaminated Central Laboratory One-Stage aPTT Assay Factor IX Activity (%) at Month 24

Cumulative Response (%) (N=54)	100	90.7	85.2	79.6	74.1	70.4	64.8	59.3	55.6	**50.0**	40.7	**31.5**	20.4	11.1	5.6	1.9
Change from Baseline in Factor IX activity	0.5	9.1	11.6	22.1	23.6	25.1	26.3	28.3	30.5	**32.5**	34.8	**42.7**	46.4	59.2	73.5	98.2

The cumulative response is defined as the percentage of subjects with a change from baseline in uncontaminated factor IX activity at Month 24 meeting or exceeding the displayed criterion.

(Source: CSR CT-AMT-061-02 24-month/Table 2.1.1.4 Sensitivity Analysis 3)

The applicant provided an updated 3-year data summary for Study CT-AMT-061-02. Briefly, the Factor IX Activity at Month 36 was 38.63 (OST-aPTT).

[redacted]

The mean uncontaminated FIX protein level was approximately 4-fold lower in Study CT-AMT-061-01 compared to Study CT-AMT-061-02 at all the time points postdose, the applicant stated this may be affected by limited number of subjects (N = 3 in CT-AMT-061-01) and absence of true baseline FIX protein values (eg, at the time of historical diagnosis). Higher within-study variability in FIX protein was seen in Study CT-AMT-061-02.

5.4.1.1.2. Bioequivalence

No dedicated biopharmaceutical studies, comparative bioavailability, or bioequivalence studies in subjects have been conducted since the qualitative and quantitative composition of etranacogene dezaparvovec formulation has not changed.

Five drug product batches were used in Study CT-AMT-061-02, and the in vitro potency range was 0.7 – 1.4 RU (Table 7). Batch A18P002 showed a slightly larger variability in FIX measurements across the time points compared to the other batches.

[redacted]

[redacted]

5.4.1.1.3. Food effect

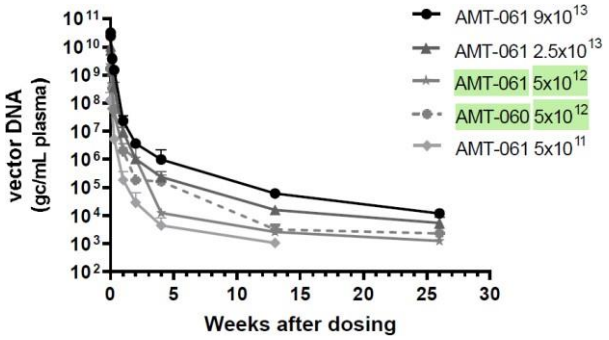
Food effect was not necessary due to intravenous infusion administration route.

5.4.1.2. Distribution

FIX protein produced by etranacogene dezaparvovec is expected to undergo similar distribution and catabolic pathways as endogenous native FIX protein. The vector DNA biodistribution result was shown in following shedding study for each clinical trial.

The biodistribution and shedding of vector DNA at different dose levels of etranacogene dezaparvovec and AMT-060 was evaluated in Cynomolgus Monkeys following a single IV dose (Report NR-061-17-001). The vector DNA plasma profiles for etranacogene dezaparvovec (AMT-061) and AMT-060 were consistent, and etranacogene dezaparvovec vector DNA levels in plasma were detectable at 6 months after injection, except the lowest dose (Figure 8).

Figure 8. Plasma Vector DNA Curves in Cynomolgus Macaques After IV Infusion with Etranacogene Dezaparvovec (AMT-061) or AMT-060

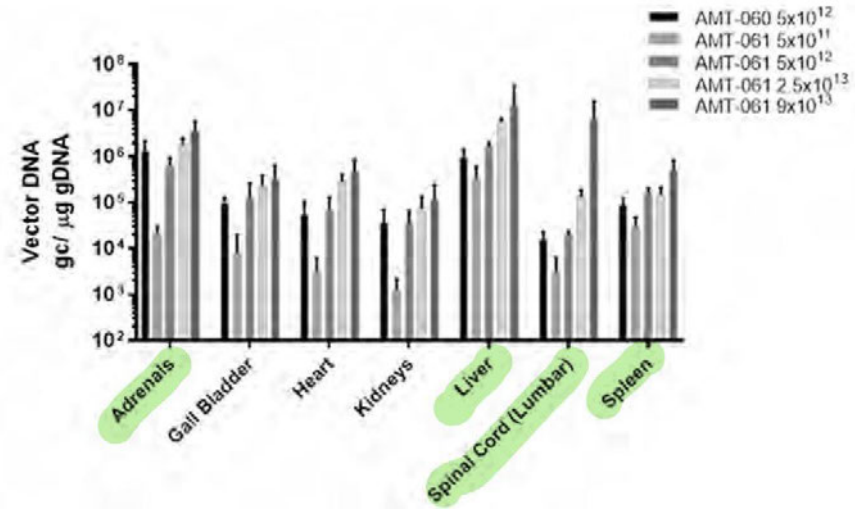


(Source: m2.6.4 Figure 6)

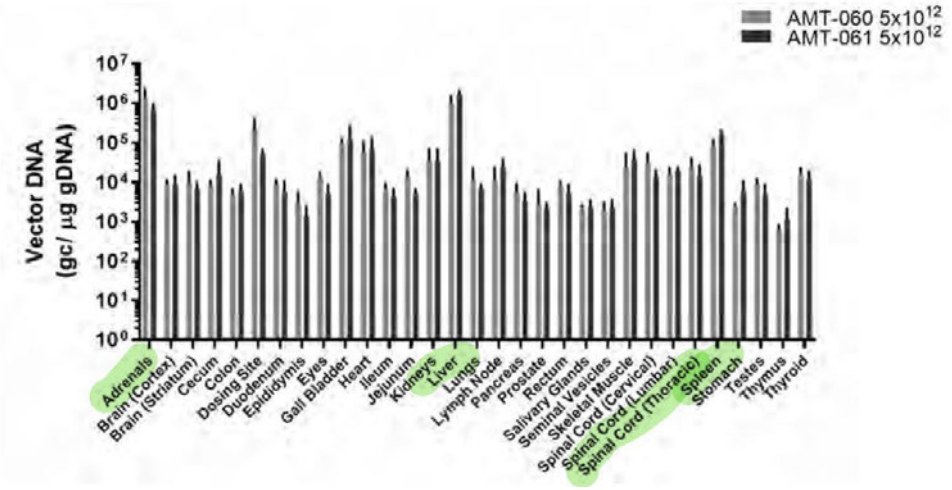
Figure 9–(A) and 9-(B) showed the vector DNA distribution in selected or all tissues at Week 26 (Report NR-061-17-001). **Vector DNA levels** were highest in the liver, and had a dose-response relationship (Figure 9–(A) and 9-(B). For the off-target tissues, adrenals, spinal cord and spleen have higher levels of vector DNA than other tissues. At the same dose (5×10^{12} gc/kg), the vector DNA levels in the evaluated organs and tissues were comparable between AMT-060 and AMT-061 (Figure 9-(B)).

Figure 9. Biodistribution of AMT-061 and AMT-060 in Cynomolgus Macaques at 26 Weeks After Infusion

A: Selected Tissues (Mean ± SD) of All Dose Groups



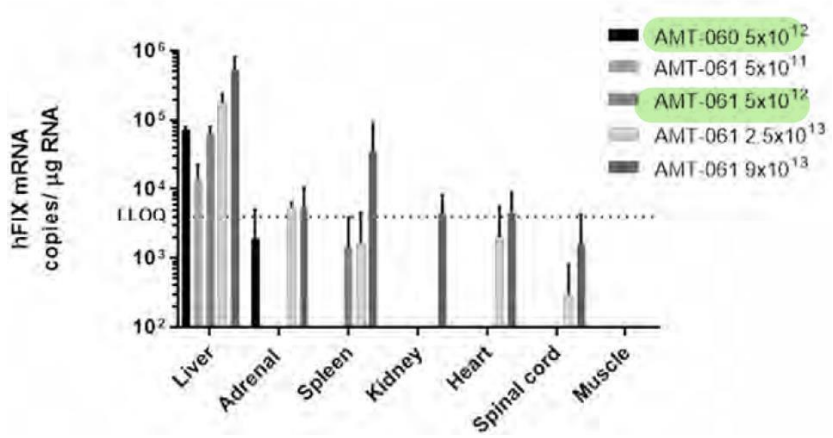
B: All Tissues (Mean ± SD) of 5×10^{12} gc/kg AMT-060 and Etranacogene Dezaparvovec Dose Groups



(Source: m2.6.4 Figure 7)

Tissue expression of vector-derived hFIX mRNA was determined by RT-qPCR (Report NR-061-17-001). The highest hFIX mRNA levels was shown in the liver, indicating the specificity (i.e. the use of a liver-specific promotor (LP1 promoter)) (Figure 10). Besides, the levels correlated with the vector dose. At the same dose (5×10^{12} gc/kg), the average **hFIX mRNA levels** were comparable for AMT-060 (7.3×10^{4} copies/µg RNA) and AMT-061(6.2×10^{4} copies/µg RNA).

Figure 10. hFIX mRNA Expression (Mean ± SD) in Selected Tissues of Cynomolgus Macaques at 26 Weeks After Infusion with AMT-061 or AMT-060



(Source: m2.6.4 Figure 8)

Biodistribution in a **paternal germline transmission study of AMT-060** was investigated in C57B1/6 mice (Report NR-060-14-001). Fifteen male animals (C57Bl/6 mice) were treated once with AMT-060 at a IV dose level of 2.3×10^{14} gc/kg, 6 days prior to pairing for mating with thirty untreated females at a 1:2 ratio. Mating day was designated day 0 of gestation **(Note: day 6 after dosing was selected, since high levels of testis vector DNA were expected)**. Males were sacrificed at 20 days post-dosing and females were sacrificed on day 17 of gestation.

Vector DNA was detected in all tissue types examined (epididymis, seminal vesicle, sperm, and testes; Table 8) from AMT-060 treated males. Vector DNA levels were below the LLOQ (1×10^2 gc/µg gDNA) in all tissue types examined from females (uterus, fetus, and placenta) following mating with treated males. No transmission of vector DNA to tissues of mated females or the F1 generation indicating absence of paternal germline transmission.

Table 8. Vector DNA Levels (Group Mean ± SD) in Reproductive Tissues

Sex	**Males**	**Females**
AMT-060 Dose (gc/kg)	**2.3×10^{14}**	**0**
FIX vector DNA (gc/µgDNA)	Mean	Mean
Epididymis	$1.3\times10^6\pm 5.6\times10^5$	NA
Seminal Vesicle	$5.2\times10^4\pm 1.8\times10^4$	NA
Sperm	$1.8\times10^6\pm 7.2\times10^5$	NA
Testis Left	$1.4\times10^6\pm 9.6\times10^5$	NA
Testis Right	$1.1\times10^6\pm 7.3\times10^5$	NA
Uterus	NA	<LLOQ
Fetus	NA	<LLOQ
Placenta	NA	<LLOQ

LLOQ: Lower Limit of Quantification (1×10^2 gc/µg DNA); NA: not applicable.

(Source: m2.6.4/Table 11, m2.6.5 Table 8)

5.4.1.3. Metabolism

No traditional metabolism study was conducted. FIX protein produced by etranacogene dezaparvovec is expected to undergo similar distribution and catabolic pathways as endogenous native FIX protein.

5.4.1.4. Excretion

No traditional mass balance study was conducted. Following presented the shedding study of each clinical trial. Overall, vector DNA from AMT-060 or etranacogene dezaparvovec administration is cleared in blood, semen, saliva, nasal secretions, urine, and feces.

[redacted]

The time to first shedding negative (i.e., the post-treatment timepoint where a negative result was measured for the first time for 3 consecutive measurements) is summarized for each sample type in Table 9 and Figure 11 (only presented blood and semen profiles herein). The mean time to reach a negative shedding result was **longest in blood, followed by in semen**, feces, saliva, nasal secretions and urine.

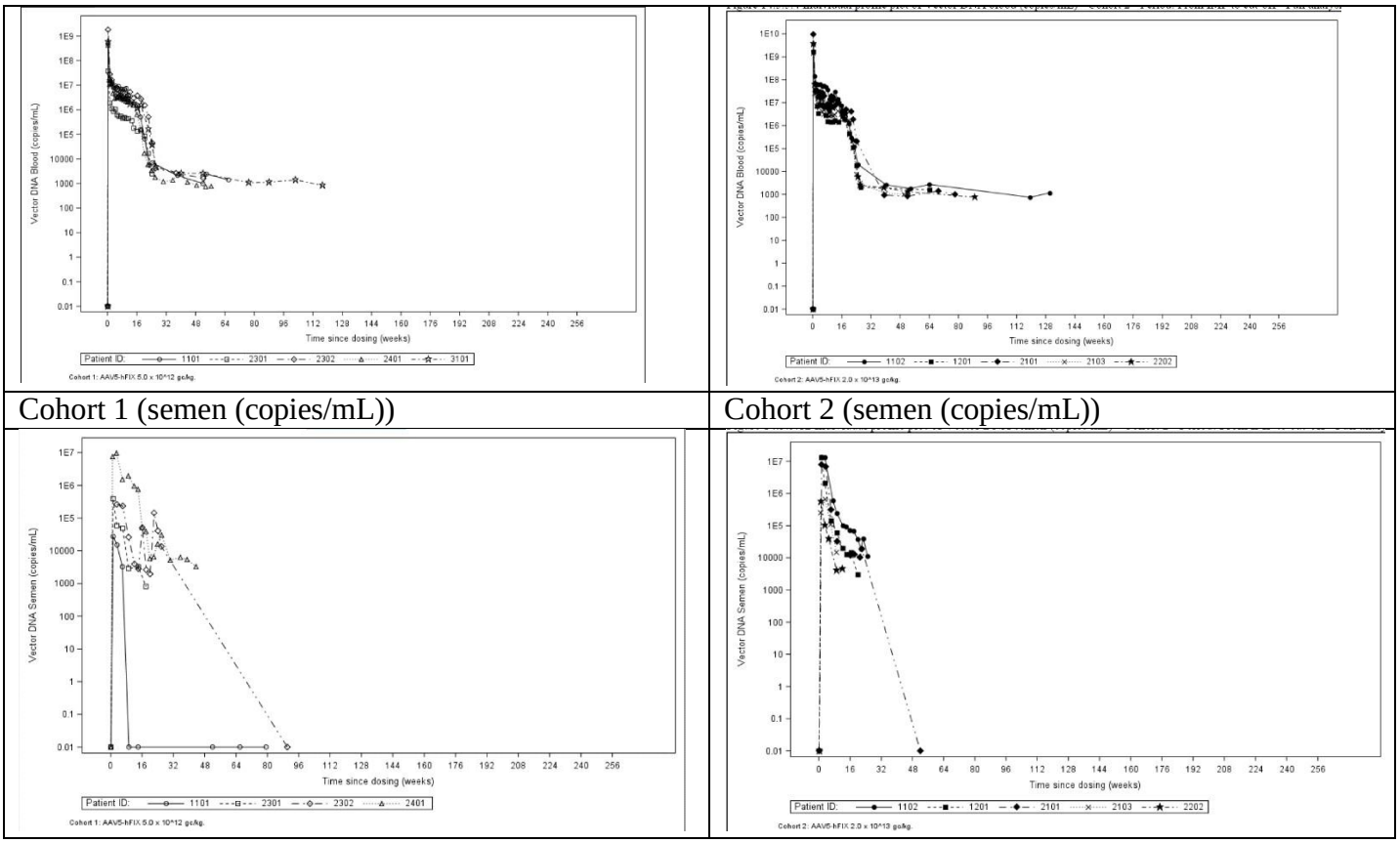
Table 9. Time to First Absence of Vector DNA Shedding in Days (FAS)

Vector DNA	Cohort 1 (N = 5)		Cohort 2 (N = 5)		Total (N = 10)	
	Mean (SD)	Min, Max	Mean (SD)	Min, Max	Mean (SD)	Min, Max
Blood (copies/mL)	508.8 (261.7)	191, 911	705.4 (245.1)	484, 1111	**607.1 (260.5)**	191, 1111
Nasal Secretions (copies/swab)	83.4 (41.7)	35, 128	108.4 (66.0)	49, 184	**95.9 (53.7)**	35, 184
Saliva (copies/mL)	75.8 (38.4)	44, 142	129.2 (48.9)	64, 182	**102.5 (50.1)**	44, 182
Urine (copies/mL)	46.4 (20.9)	23, 79	82.0 (41.1)	57, 155	**64.2 (36.0)**	23, 155
Feces (copies/mg, n)	74.0 (25.7)	43, 111	165.0 (68.9)	112, 282	**119.5 (68.6)**	43, 282
Semen (copies/mL)	227.8# (147.7)	65, 365	157.2 (78.4)	84, 282	**188.6 (112.4)**	65, 365

#: All data were from 5 individual subjects, except semen data in in Cohort 1 was from 4 subjects. Since one subject (Subject 3101) was unable to produce semen due to a historical medical condition and, therefore, shedding from semen could not be assessed.
(Source: m2.7.2 Table 4; CSR CT-AMT-060-01/Table 28 and Table 14.3.7.2)

Figure 11. Individual profile plot of Vector DNA - Cohort 1 and Cohort 2

Cohort 1 (blood (copies/mL))	Cohort 2 (blood (copies/mL))



Cohort 1 (semen (copies/mL)) | Cohort 2 (semen (copies/mL))

(Source: CSR CT-AMT-060-01/ Figure 14.3.5.3, Figure 14.3.5.4, Figure 14.3.5.11 and Figure 14.3.5.12)

[redacted]

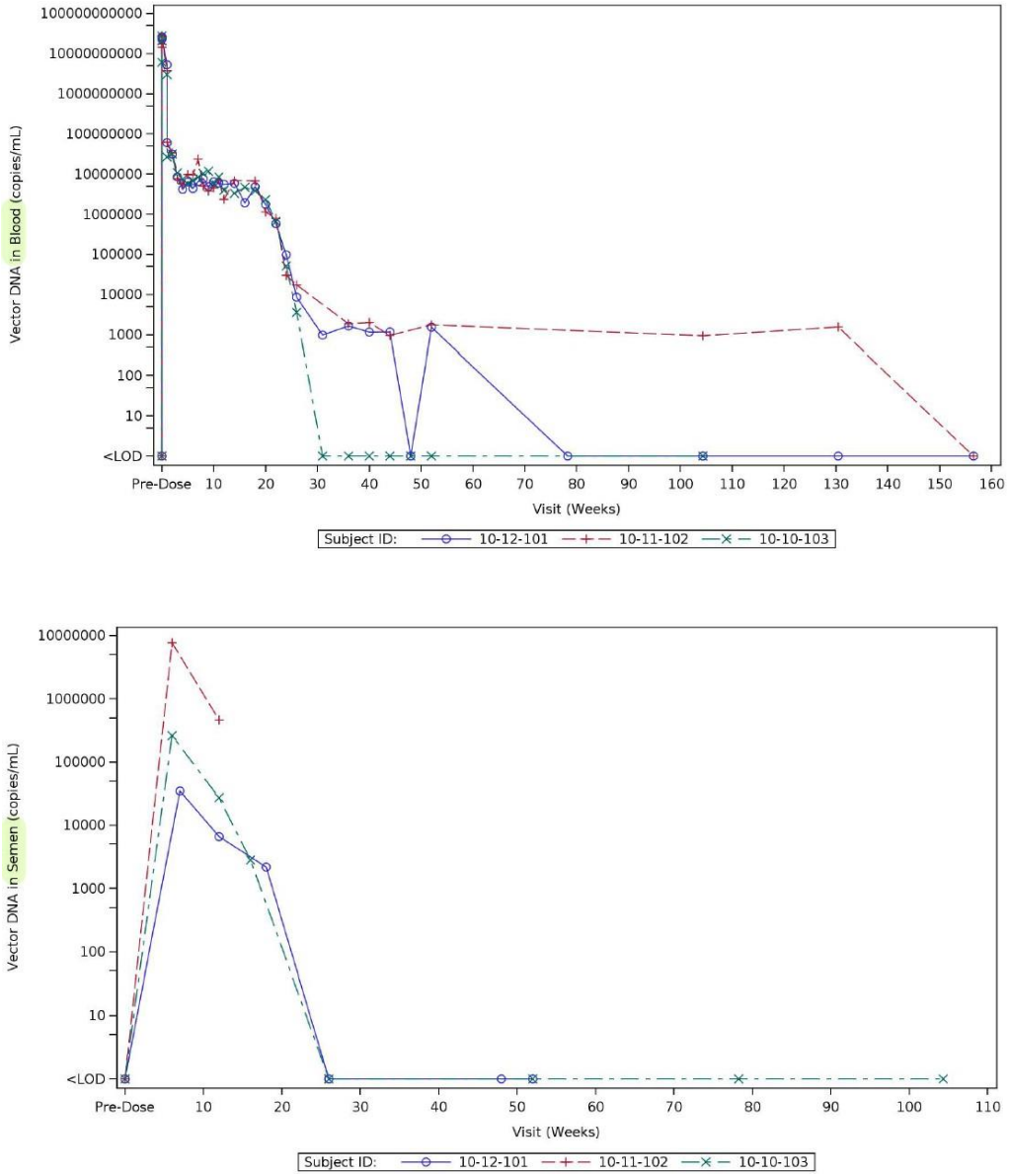
Figure 12 and Table 10 presented the results of AAV vector DNA shedding in semen or blood. Two (2) subjects attained the status of no longer shedding vector DNA at a mean of 26.21 weeks for semen samples and at 54.71 weeks for blood samples. The other one [redacted] still had positive test results at all postdose visits up to Month 30 and a negative test result at Month 36.

Table 10. Adeno-Associated Virus (AAV) Vector DNA Shedding - Number of Weeks to Negative Analysis Set: All Subjects Treated

Statistics	**First Week Negative (N=3)**	
	Blood (n=2)	**Semen (n=2)**
Mean (SD)	54.71 (33.34)	26.21 (0.10)
Median	54.71	26.21
Minimum, Maximum	31.1, 78.3	26.1, 26.3
For semen: [redacted] and [redacted] had the first of 3 consecutive negative results at Week 26. [redacted] had results post-Baseline for Week 6 and Week 12 (both positive). **For blood:** [redacted] had the first of 3 consecutive negative results at Week 31. [redacted] had a negative result at Week 48 but tested positive at Week 52; results from subsequent visits were negative (Month 18 on). Subject 10-11-102 had positive test results at all visits post-AMT-061 administration up to Month 30 and a negative test result at Month 36.		

(Source: CSR CT-AMT-061-01/Table 3.4.1)

Figure 12. Individual Plot of AAV Vector DNA in Semen (copies/mL) or Blood Over Time [Period: From IMP to Cut-Off ; Analysis Set: All Subjects Treated]

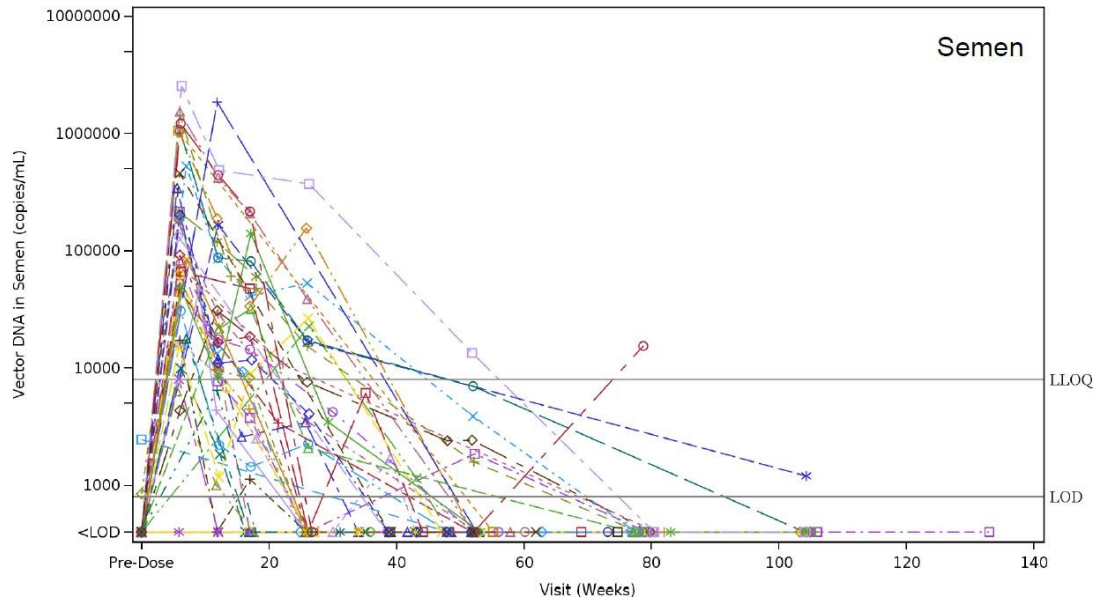


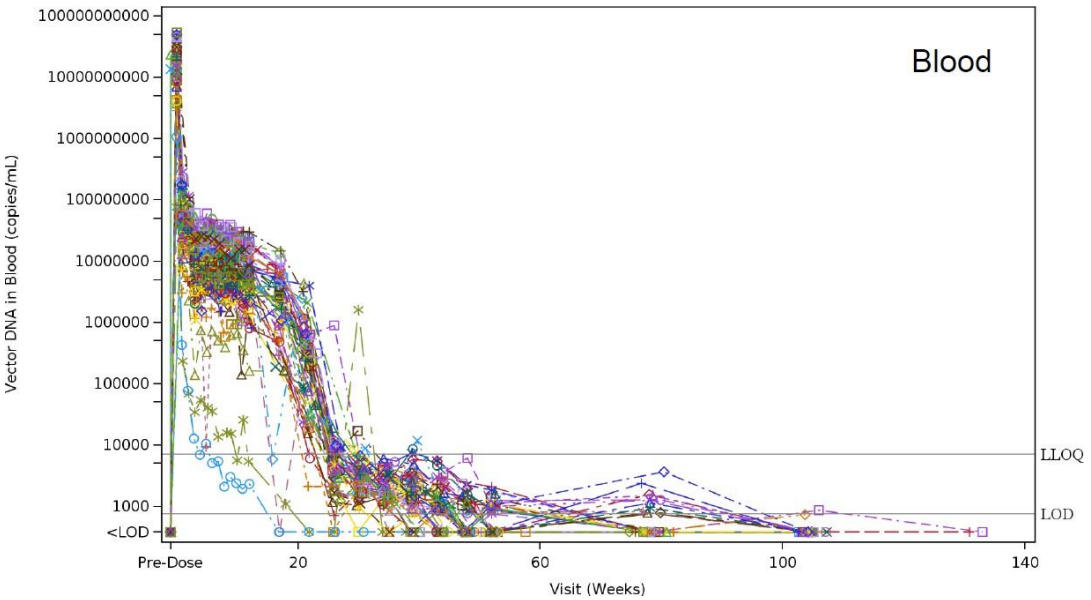
(Source: CSR CT-AMT-061-01/Figure 3.5.1 and Figure 3.5.2)

[redacted]

Based on individual data, the time of maximum levels of vector DNA in blood and semen was observed between 4 and 7 hours and between Weeks 5 and 27, respectively (Figure 13).

Figure 13. Individual Plot of AAV Vector DNA in Semen (copies/mL) or Blood (copies/mL) Over Time –[Period: From IMP to Cut-Off Analysis; Set: Post-Treatment Safety Population]





- Semen: Lower limit of quantification (LLOQ)=8000. Lower limit of detection (LOD) = 800.
- Blood: Lower limit of quantification (LLOQ)=7060. Lower limit of detection (LOD) = 760.

(Source: CSR CT-AMT-061-02/ Figure 3.5.1 and 3.5.2)

In the post-AMT-061 treatment period, clearance of vector DNA from semen or blood was confirmed in 32/54 (59.3%) and 30/54 (55.6%) subjects, respectively. The earliest that subjects were considered to be no longer shedding vector DNA from semen and blood was 6 weeks (1.9% of subjects) and 17 weeks (1.9% of subjects), after treatment with etranacogene dezaparvovec. Median time to vector clearance in semen and blood was 47.3 weeks and 52.3 weeks.

Table 11. AAV Vector DNA Shedding During the Post-treatment Period (Safety Population)

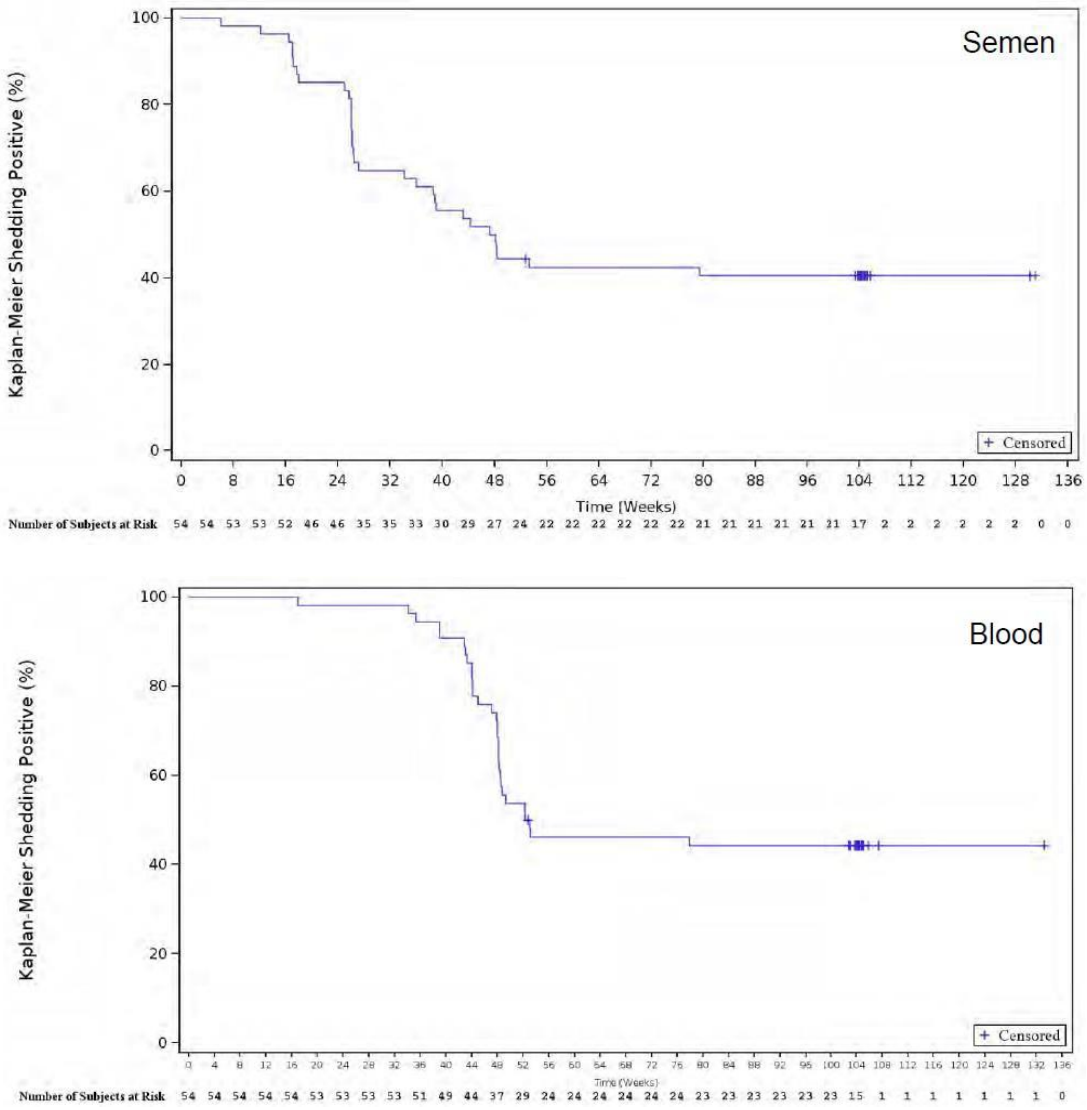
n (%)	Semen (N = 54)	Blood (N = 54)
Attained Vector Shedding Negative, n (%)	32 (59.3)	30 (55.6)
Time to Vector Shedding Negative[1]		
P10 (95% CI) (weeks)	17.1 (12.1, 26.0)	42.9 (34.1, 44.1)
P25 (95% CI)	26.0 (18.0, 36.0)	47.1 (43.3, 48.3)
Median (95% CI)	47.3 (36.0, NE)	52.3 (48.3, NE)

Pxx denotes the xx-th percentile.

1. Time in weeks until first "LOD" (limit of detection) in consecutive order of 3 or more timepoints with a negative result. This is the time until "shedding negative". The statistics were computed by the Kaplan-Meier method. 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.

(Source: CSR CT-AMT-061-02/Table 39; 24-month Table 3.7 and Table 3.8)

Figure 14. Kaplan-Meier Curve for Time to First Vector Shedding Negative From Semen or 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.
(Source: CSR CT-AMT-061-02/Figure 6 and 7; 24-month Figure 3.5.1 - Figure 3.5.4)

The applicant provided an updated information for shedding of vector DNA for Study CT-AMT-061-02 (Year 3 summary report). Briefly, the updated shedding test was described as follows.

[redacted]

- A total of 46/54 (85.2%) subjects attained the status of no longer shedding vector DNA from blood at the time of the 3-year database extract.
- The earliest that subjects were considered to no longer be shedding vector DNA from blood was 17 weeks after treatment with CSL222 (1.9% of subjects [95% CI: 0.3, 12.4]).
- Median time to absence of shedding was 52.6 weeks (95% CI: 48.1, 77.9; CT-AMT-061-02 3-year Table 3.8).
- 8/54 subjects are not yet defined as "shedding negative". One of 8 subject died due to a non-treatment-related cause, but his/her last sample at Month 12, with a vector DNA level < LLOQ.

- Semen:
  - A total of 45/54 (83.3%) subjects attained the status of no longer shedding vector DNA from semen at the time of the 3-year database extract.
  - The earliest that subjects were considered to no longer be shedding vector DNA from semen was 6 weeks after treatment with CSL222 (1.9% of subjects [95% CI: 0.3, 12.4]).
  - Median time to absence of shedding was 43.7 weeks (95% CI: 34.1, 51.9; CT-AMT-061-02 3-year Table 3.7).

5.4.2. Pharmacodynamics and Pharmacokinetic-Pharmacodynamic correlation

Please refer to the above FIX activity data.

5.5. Special populations

5.5.1. Effects of Intrinsic and Extrinsic Factors on Uncontaminated FIX Activity

Since the absence of true baseline for FIX protein (eg. at the time of historical diagnosis) in etranacogene dezaparvovec clinical studies, presence of endogenous less-functional or non-functional FIX protein, and large variability associated with the FIX protein measurement, no reliable data can be used to interpret the impact of intrinsic or extrinsic factors on FIX protein.

The intrinsic or extrinsic factors on FIX activity werer discussed as follows.

- **Sex:** All subjects included in clinical trials were male, gender effect could not be characterized.

[redacted]

Visit	FIX Activity (%)		
	< 40 years (N = 31)	40-60 years (N = 15)	≥ 60 years (N = 7)
Week 6, n	26	15	6
Mean (SD)	27.95 (11.571)	36.13 (14.841)	42.02 (20.652)
Month 3, n	29	15	7
Mean (SD)	31.47 (14.246)	42.94 (20.391)	45.61 (22.610)
Month 6, n	29	15	7
Mean (SD)	33.09 (15.669)	45.37 (19.428)	49.46 (22.272)
Month 12, n	29	14	7
Mean (SD)	34.40 (18.349)	49.40 (21.137)	54.96 (26.657)
Month 18, n	29	15	6
Mean (SD)	30.20 (14.192)	39.31 (15.708)	63.28 (39.424)

[redacted]

The applicant provided the new CT-AMT-061-02/ad hoc Table 128.11 to show the FIX activity levels (OSC-aPTT) stratified by age and ALT elevation. It can see that there still appears to be a trend of higher FIX activity with increase in age. For the < 40 age group, there does not appear to be a difference in the mean FIX activity levels when stratified by ALT elevation.

Since ALT elevation can decrease the FIX activity, the high variable in FIX protein and FIX activity and small sample size of subjects, the explanation from the applicant can be accepted.

[redacted]

[redacted]

Since the subjects enrolled in all clinical trials were dosed based on actual body weight, and no significant highest FIX activity levels in highest BMI subgroup; thus, it can accept that etranacogene dezaparvovec can be dosed based on actual body weight.

- **Effect of Postdose ALT Elevation:** ALT elevation was defined as subjects with (1) ALT > ULN based on central lab or local lab when the baseline ALT is below ULN, or (2) Postdose ALT value of > 2 × the baseline ALT level measured by either central or local laboratory, occurring within the initial 90 days post etranacogene dezaparvovec administration.
  Twenty-five percentage (25%; 13/53) of subjects had ALT elevation. Almost all of these were mild Grade 1 events (one Grade 3 event). The mean FIX activity levels in the subjects with ALT elevation were lower than that in those without ALT elevation [6 months: ↓ 37% ; 12 months: ↓ 46% , 18 month: ↓ 44% , and 24 months: ↓ 41%. Table 14]. FIX activity over time in subjects with and without ALT elevation was shown in Figure 15.

Table 14. Effect of ALT Elevation by Visit on Uncontaminated FIX Activity of Etranacogene Dezaparvovec (PK Population)

	FIX Activity (%)

Visit	With ALT Elevation > ULN (N = 13)	Without ALT Elevation > ULN (N = 40)
Week 1	22.53 (17.7, 26.0)	26.60 (20.2, 35.8)
Week 2	24.34 (18.2, 31.8)	23.49 (11.6, 49.1)
Week 3	19.08 (4.9, 34.1)	29.18 (6.7, 56.7)
Week 4	23.75 (7.2, 46.7)	31.16 (7.7, 66.5)
Week 5	22.72 (7.5, 52.7)	31.32 (1.5, 61.5)
Week 6	23.71 (6.4, 57.4)	34.69 (16.2, 81.3)
Week 9	28.24 (9.1, 50.9)	37.13 (12.0, 88.9)
Month 3	25.48 (7.6, 50.2)	40.26 (9.1, 91.0)
Month 6	26.80 (8.2, 68.6)	42.69 (13.8, 97.1)
Month 9	24.83 (5.9, 63.8)	45.53 (11.9, 118.0)
Month 12	25.23 (5.9, 68.1)	46.61 (13.2, 113.0)
Month 18	23.15 (4.5, 61.8)	41.24 (12.0, 122.9)
Month 24	24.03 (4.7, 60.4)	40.64 (9.1, 99.2)

ALT= alanine aminotransferase

(Source: m2.7.2 Table 18; 24-month Durability and PK Analysis, Table 1.11.)

[redacted]

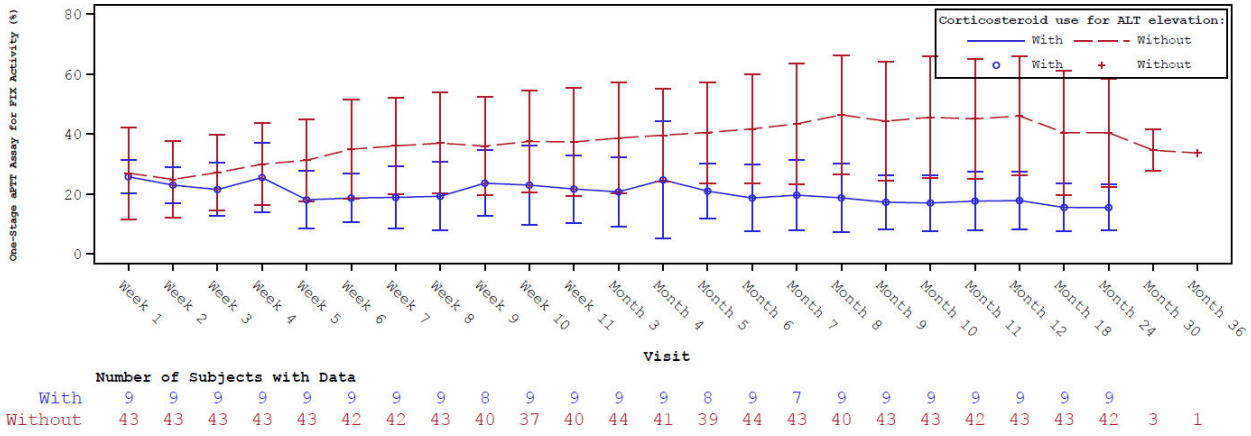
- **Effect of Corticosteroid Use:** Corticosteroids were administered to subjects for increases in ALT elevation. A total of 9 out of 53 subjects received corticosteroids for ALT elevation. These subjects had lower mean FIX activity levels by 40% - 64% than that in the rest of the 44 subjects (Table 15). FIX activity over time by corticosteroid use for ALT elevation was shown in Figure 16. Five subjects had ALT elevations but did not receive steroids. Of these 5 subjects, 4 had > 18% of normal FIX activity at all time points postdose and only one subject had lower FIX activity between 6% and 10% of normal FIX activity during the post treatment period.

Table 15. Uncontaminated FIX Activity (%) in Subjects With and Without Corticosteroid Use for ALT Elevation in Study CT-AMT-061-02(PK Population)

Visit	FIX Activity (%)	
	Corticosteroid Use for ALT Elevation in Study CT-AMT-061-02	
	Yes (N = 9)	All Other Subjects (N = 44)
Week 6, n	7	40
Mean (SD)	19.51 (8.980)	34.61 (14.323)
Week 9, n	7	38
Mean (SD)	22.71 (11.561)	37.68 (15.059)
Month 3, n	8	43
Mean (SD)	21.83 (11.768)	39.57 (17.871)
Month 6, n	9	42
Mean (SD)	18.72 (11.086)	43.29 (17.155)
Month 9, n	9	42
Mean (SD)	17.31 (9.029)	45.17 (19.416)
Month 12, n	8	42
Mean (SD)	16.70 (9.696)	46.20 (20.118)
Month 18, n	9	41
Mean (SD)	15.56 (7.920)	41.59 (20.573)
Month 24, n	9	41
Mean (SD)	15.52 (7.710)	41.30 (17.486)

ALT= alanine aminotransferase
(Source: m2.7.2 Table 18; 24-month Durability and PK Analysis, Table 1.7)

Figure 16. Mean (+/- SD) of FIX activity over time **by corticosteroid use for ALT elevation** following administration of Etranacogene Dezaparvovec PK Analysis Population



(Source: 24-month Durability and PK Analysis, Figure 1.1)

5.5.2. Renal impairment

No dedicated renal impairment study was conducted. The subjects in Study CT-AMT-061-02 were classified to have normal (CrCl: ≥ 90 ml/min), mild (CrCl:60 – 89 ml/min) or moderate (CrCl:30 – 59 ml/min) renal impairment (RI) based on the Cockcroft-Gault equation. No subjects with severe renal impairment or kidney failure (end stage renal disease) were enrolled.

From week 6 to Month 24, it was higher by 24% - 42% in mild RI subgroup, compared to subjects with normal renal function. Also, the only one subject with moderate RI have comparable FIX activity (±22%).

The proposed posology in Chinese labeling was no dose adjustment was required in mild or moderate RI patients. PK reviewer though it is acceptable since (1) etranacogene dezaparvovec is a gene therapy drug product, (2) no other recombination-FIX or blood coagulation Factor IX drug product mentioned the dose adjustment for RI, even though the number of subjects in moderate RI is limited.

Table 16. Effect of Baseline Renal Impairment on Uncontaminated FIX Activity (%) of Etranacogene Dezaparvovec (PK Population)

Visit	FIX Activity (%)		
	Baseline Renal Impairment Category:		
	Normal (N = 45)	Mild (N = 7)	Moderate (N = 1)
Week 6, n	40	7	-
Mean (SD)	31.25 (13.309)	38.69 (20.833)	-
Month 3, n	43	7	1
Mean (SD)	35.55 (17.377)	44.90 (23.384)	32.80 (-)
Month 6, n	43	7	1
Mean (SD)	37.12 (17.749)	50.94 (22.775)	33.80 (-)
Month 12, n	42	7	1
Mean (SD)	39.38 (20.348)	53.17 (28.696)	47.90 (-)
Month 18, n	43	6	1
Mean (SD)	35.21 (20.436)	48.07 (28.252)	42.50 (-)
Month 24, n	43	6	1
Mean (SD)	34.90 (17.570)	49.63 (26.430)	34.20 (-)

(Source: m2.7.2 Table 14, 15; 18-month Durability and PK Analysis, Table 1.6)

5.5.3. Hepatic impairment

No dedicated hepatic impairment study was conducted. The analysis used Baseline FibroScan™ or equivalent shear wave elastography, magnetic resonance elastography to determine baseline liver pathology, and classified as: (1) Degree of fibrosis (≥ 9 kilopascals [kPa] versus < 9 kPa); (2) Degree of steatosis (CAP score ≥ S2 [≥ 260 dB/m] versus < S2 [< 260 dB/m] versus Missing).

All subjects enrolled in Study CT-AMT-061-02 had < 9 kPa degree of fibrosis; none had ≥ 9 kPa. No further assessment was acquired.

Three (3) steatosis subgroups (≧S2, < S2 and missing) were classified based on CAP score. No clinically meaningful differences in FIX activity (< 30% relative difference between < S2 and ≥ S2 groups) were observed (Table 17).

Table 17. Effect of Baseline Liver Pathology by Steatosis Grade Category on Uncontaminated FIX Activity (%) of Etranacogene Dezaparvovec (PK Population)

FIX Activity (%)	Baseline Steatosis Grade Category		
Visit	≥ S2 (N = 11)	< S2 (N = 28)	Missing (N = 14)
Week 6	26.01 (6.4, 46.5)	34.03 (10.5, 81.3)	34.90 (18.1, 65.9)
Month 3	31.72 (7.6, 52.4)	39.77 (10.7, 91.0)	35.22 (9.1, 89.2)
Month 6	34.52 (10.0, 52.7)	40.79 (8.2, 97.1)	38.57 (13.0, 90.4)
Month 12	32.55 (5.9, 60.5)	46.38 (11.4, 113.0)	38.76 (12.8, 73.6)
Month 18	29.22 (4.5, 49.3)	41.57 (10.3, 122.9)	33.12 (12.0, 57.9)
Month 24	28.38 (4.7, 47.4)	40.16 (10.1, 99.2)	35.75 (9.1, 88.3)

Mean (Min, Max).; ≥ S2 : ≥ 260 dB/m; < S2: < 260 dB/m.

(Source: m2.7.2 Table 13; 18-month Durability and PK Analysis, Table 1.5.2.)

The applicant stated that the degree of steatosis was only captured as S2 or < S2, so no further subdivisions of the S2 group is possible. Since this analysis is not a critical issue; thus, lack of this information can be acceptable.

5.6. Drug-drug interaction

No dedicated DDI study was conducted.

5.7. Consideration of intrinsic factors

Of 57 subjects enrolled in Studies CT-AMT-061-01 and CT-AMT-061-02, 52 subjects reported race by themselves, including 41 (78.8%) White and 11 (21.2%) non-White. Of the 11 non-White subjects, 2 (3.8%) were Asian, 3 (5.8%) were Black or African American, and 6 (11.5%) were other.

Table 18 showed the race impact on the FIX activity in Study CT-AMT-061-02. The applicant stated that no notable differences (< 30% relative difference) were observed in FIX activity in White (40/53, 75%) versus Non-White subjects at Month 6 to 18 post-dose. However, the FIX activity in Asian subjects (n=2) was lack. More data was needed to provide.

Table 18. Effect of Race on Uncontaminated FIX Activity (%) of Etranacogene Dezaparvovec (Study CT-AMT-061-02, PK Population; One-Stage aPTT)

Visit	Non-White (N=8)	White (N=40)
Week 6	26.82 (20.3, 46.9)	33.24 (6.4, 81.3)
Month 3	27.03 (9.1, 46.7)	38.67 (7.6, 91.0)
Month 6	29.07 (13.8, 44.3)	41.04 (8.2, 97.1)
Month 12	31.84 (13.2, 48.2)	43.01 (8.5, 113.0)
Month 18	28.43 (12.0, 51.8)	39.07 (10.3, 122.9)
Month 24	28.89 (9.1, 51.7)	38.92 (10.1, 99.2)

Mean (Min, Max).

(Source: m5.3.5.3 18-month Durability and PK Analysis, Table 1.3)

The results of FIX activity level (based on OST and CS), vector DNA shedding and immunogenicity in Asian subjects (n=2) were provided, and PK reviewer reorganized the data and presented in following Table. Since the race and ethnicity were self-reported by subjects, the applicant did not know whether they belonged to East Asian or not.

Based on the following data, the FIX activity (%) of 2 Asian subjects were within the range of FIX activity (%) for White (N=40; refer to Table 18). For vector DNA shedding, both subjects were defined as "shedding negative" at Month 6 for semen and Month 18 for blood. For immunogenicity, both subjects had higher than average baseline NAb titers compared with the non-Asian population, and both titers were in the 4th quartile of all subjects.

	Subject No. (Study CT-AMT-061-02)			
	[redacted]		[redacted]	
FIX Activity (%) **Visit**	**One-Stage aPTT***	**CS#**	**One-Stage aPTT***	**CS#**
Baseline	1.0	1.0	2.0	2.0
Scerrning	23.0	-	23.5	-
Lead-in final	64.0	61.5	19.3	9.2
[redacted]	16.0	8.3	21.0	10.6
Week 3	18.0	8.3	20.5	9.6
Week 6	26.0	11.2	23.0	11.2
Month 3	29.0	11.7	24.5	9.4
Month 6	25.0	12.7	24.2	11.5
Month 12	28.0	11.8	16.0	-
Month 18	26.0	14.1	17.0	11.9
Month 24	31.0	14.9	28.9	14.8
Month 30	30.0	14.7	-	-
[redacted]	Blood	Semen	Blood	Semen
	At Month 18	Month 6	At Month 18	Month 6

One-Stage: aPTT Listing 2.1.1; Chromogenic:Listing 2.1.2.

*: Lab name - Local. : Lab name – Unilabs.

(Source; PK supplement 5_藥動部分(附件 13)/ 1_(一)2 位亞洲受試者; The listings for FIX activity levels for the one-stage assay (Listing 2.1.1), the chromogenic assay (Listing 2.1.2), FIX protein levels (Listing 2.1.3), vector DNA – blood (Listing 3.8.1) and vector DNA – semen (Listing 3.8.2))

Overall, the exact race (East Asian) effect on the PK and PD of etranacogene dezaparvovec can not be concluded due to lack of the information about race and ethnicity. However, the ethnic difference can be considered negligible because of the nature of the gene product.

5.8. External Communication (deficiency/inquiry and supplement, expert consultation…etc.)

The applicant's responses to the supplementary information have been included in the relevant chapters of the report.

5.9. **Conclusion and Recommendation**

Overall, the pharmacokinetic studies conducted were satisfactory met the minimum requirements to support the marketing authorization of Hemgenix. It is recommended to approve the NDA of Hemgenix from the PK/PD perspective.

6. Statistical Evaluation
6.1 Introduction

This is a New Drug Application of Hemgenix® 1×10^{13} genome copies/mL (Etranacogene dezaparvovec). Hemgenix® is indicated for treatment of adults with Haemophilia B (congenital Factor IX deficiency) and with a preexisting neutralising AAV5 antibody titre below 1:900. The dose of etranacogene dezaparvovec is a single dose of 2×10^{13} gc per kilogram (kg) of body weight (bw) or 2.0 mL/kg bw, administered as an intravenous infusion after dilution with 0.9% sodium chloride solution (normal saline).

In this submission, the Sponsor provided a phase III study, CT-AMT-061-02, to support the efficacy of Hemgenix® for the claimed indication. The major design features of the study were summarized in Table 6.1-1.

Table 6.1-1: Major design features of CT-AMT-061-02

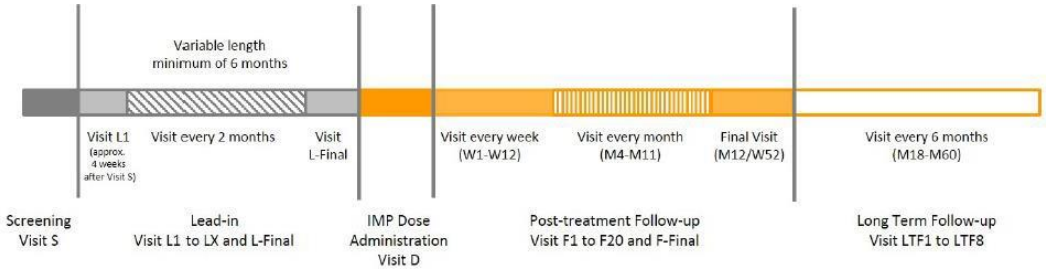
Design	Population	Treatment group	Primary endpoint	Statistical Method
Open-label single-dose multi-center multi-national	Subjects were adult males with severe or moderately severe hemophilia B.	Subjects would receive a single IV infusion of 2×10^{13}gc/kg AMT-061.	Annualized Bleeding Rate (ABR)	Estimated rate ratio, one-sided 97.5% Wald CI and the corresponding p-value

6.2 Study designs
CT-AMT-061-02

CT-AMT-061-02 is an open-label, single-dose, multi-center, multi-national trial, with a screening period, a lead-in phase, a treatment plus a post-treatment follow-up phase, and a long-term follow-up phase.

During the lead-in phase, which lasted for a minimum of 26 weeks (i.e. ≥6 months), subjects recorded their use of FIX replacement therapy and bleeding episodes in their dedicated e-diary. After the lead-in phase, subjects received a single-dose of AMT-061 at the dosing visit (Visit D) and were followed for 1 year to evaluate efficacy and safety. Overview of the trial design is presented in Figure 6.2-1.

Figure 6.2-1: Study Design of CT-AMT-061-02

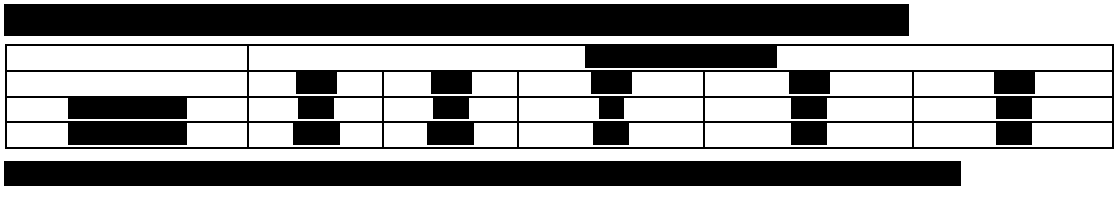


The primary objective of study CT-AMT-061-02 was to demonstrate the non-inferiority of AMT-061 (2×10^{13} gc/kg) during the 52 weeks following establishment of stable factor IX expression (months 6 to 18) post treatment (AMT-061) follow-up compared to standard of care continuous routine factor IX prophylaxis during the lead-in phase.

The primary endpoint was Annualized Bleeding Rate (ABR) comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable factor IX expression (months 6-18 post-treatment). The unadjusted ABR rates were estimated as 365.25 x (number of bleeding episodes)/ (uncontaminated person-time in the observed treatment period of interest).

For the primary analysis, analysis of the number of reported bleeding events was performed using a repeated measure generalized estimating equations (GEE) negative binomial regression model accounting for the paired design of the trial with an offset parameter to account for the differential collection periods. The model included the treatment (i.e. period) as a categorical variable. The estimated rate ratio and one-sided 97.5% Wald CI and the corresponding p-value were 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 was less than 1.8, then non-inferiority would be declared.

The applicant adopted two approaches to obtain the non-inferiority margin (Table 6.2-1). The NI margin of 1.8 corresponds to a 59% retention of treatment effect in approach (2).



Sensitivity analyses of the primary efficacy endpoint included PP population, bleeds treated with exogenous factor IX, and new and true bleeds treated with exogenous factor IX.

Key secondary efficacy endpoints were tested using a hierarchical approach, which continued until a non-significant result was obtained. The order of fixed sequential tests was specified below:

1. Endogenous FIX activity at 6 months after AMT-061 dosing
2. Endogenous FIX activity at 12 months after AMT-061 dosing
3. Endogenous FIX activity at 18 months after AMT-061 dosing
4. Annualized consumption of FIX replacement therapy during the 52 weeks following stable FIX expression (6 to 18 months) post-treatment follow-up, excluding FIX replacement for invasive procedures, compared to the lead-in phase
5. Annualized infusion rate of FIX replacement therapy during the 52 weeks following stable FIX expression (6 to 18 months) post-treatment follow-up, excluding FIX replacement for invasive procedures, compared to the lead-in phase
6. 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 post-treatment)
7. ABR comparison between AMT-061 and prophylaxis for superiority between the lead-in phase and the 52 weeks following stable FIX expression (6 to 18 months) post-treatment
8. Rate of spontaneous bleeding episodes during the 52 weeks following stable FIX expression (6 to 18 months) post-treatment compared to lead-in phase
9. Rate of joint bleeding episodes during the 52 weeks following stable FIX expression (6 to 18 months) post-treatment compared to the lead-in phase
10. 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
11. PRO questionnaire scores from the EQ-5D-5L VAS score during the 12 months following AMT-061 dosing compared with the lead-in phase

The efficacy analysis would be performed using the Full Analysis Set (FAS). The FAS included all subjects who were enrolled, entered the lead-in phase, were dosed with AMT-061, and provide at least one efficacy endpoint assessment for any efficacy endpoint subsequent to AMT-061 dosing.

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

Reviewer's Comments:

There is no critical deficiency observed in the study design and statistical analysis in this study.

6.3 Results (main results, key subgroups)
Study CT-AMT-061-02
Patient disposition

A total of 75 subjects were screened and 67/75 (89.3%) subjects entered the lead-in period (Table 6.3-1). There were 54/67 (80.6%) subjects treated with AMT-061, of which 53/54 (98.1%) subjects completed treatment. One subject prematurely discontinued treatment infusion due to an AE of hypersensitivity and received a partial dose (10%); the subject continued in the study for follow-up.

Table 6.3-1: Summary of Subject Disposition

		Total (N = 75) n (%)
Entered Lead-in Period		67 (89.3)
Treated with AMT-061		54 (80.6)
	Prematurely Discontinued from Treatment due to Adverse Event (Received Partial Dose)	1/54 (1.9)
	Completed Treatment (Received Full Dose)	53/54 (98.1)
	Early Withdrawal from Study (Post-treatment)	2/54 (3.7)
	Adverse Event	1/2 (50.0)
	Subject Withdrew Consent	1/2 (50.0)

* The PROBE sub-study was the subset of the FAS that had at least one post-treatment assessment of the given assessment tool.

Demographic and baseline characteristics

All subjects were male. The majority of subjects enrolled in the Post-treatment Safety Population (FAS) were White (74.1%) and not Hispanic or Latino (83.3%; Table 6.3-2). Subjects in the Post-treatment Safety Population had a mean (SD) age of 41.5 (15.8) years, mean (SD) height of 176.5 (8.2) cm, mean (SD) weight of 85.1 (19.3) kg, and mean (SD) BMI of 27.2 (5.1) kg/m^2.

Table 6.3-2: Summary of Demographic and Baseline Characteristics

Characteristic	Lead-in Safety Population (N = 67)	Post-treatment Safety Population/FAS (N = 54)	PP Population (N = 53)
Age (years), n	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)
Min, Max	19, 78	19, 75	19, 75
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

Efficacy

- ***Primary efficacy endpoint:***

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

The mean 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). The adjusted ABR rate ratio for the Month 7 to 18 post-treatment period to lead-in period was 0.36 (95% Wald CI: 0.20, 0.64; p=0.0002). 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. In addition, the superiority of AMT-061 over standard of care was achieved.

Reviewer's Note:

1. *The applicant had different definitions of the time of the primary endpoint in the protocol (months 6-18 post-treatment) and CSR (months 7-18 post-treatment).*
2. *The applicant interpreted the primary endpoint was analyzed as the number of bleedings reported starting from Month 7 or Day 183 postdose (ie, after the 6-month period allowing for the stabilization of FIX) until Month 18. Although the*

Postdose Period was stated differently in the protocol and CSR, the meaning remained the same. The period from Month 7 to Month 18 postdose was considered more intuitive and accurately reflected the actual analysis period.

- ***Sensitivity analyses:***

Sensitivity analyses included PP population, bleeds treated with exogenous factor IX, cumulative responder analysis using subject-specific bleeding rates, and new and true bleeds treated with exogenous factor IX. Results showed that sensitivity analyses demonstrated the robustness of the ABR results.

Table 6.3-3: Sensitivity analyses of the primary efficacy endpoint

	Rate Ratio	95% CI	P-value
PP population	0.33	0.19. 0.60	0.0001
Bleeds treated with exogenous factor IX (FAS)	0.23	0.12, 0.46	<0.0001
New and true bleeds treated with exogenous factor IX (FAS)	0.19	0.09, 0.41	<0.0001

- ***Subgroup analysis:***

ABR was significantly reduced during Months 7 to 18 and Months 7 to 24 after AMT-061 treatment compared to the lead-in period for most of the subgroups analyzed, with rate ratios (post treatment/ lead-in) ranging from 0.16 to 0.57 and 0.10 to 0.92, respectively ($p < 0.025$ for most subgroups [not adjusted for multiplicity]).

We found that estimates of unadjusted ABR and adjusted ABR were very different for race group and status of target joint at screening category group (Table 6.3-4).

Table 6.3-4: Partial Subgroup Analysis of Annualized Bleeding Rates (Full Analysis Set)

	≥6-month Lead-in Period		Month 7-18			Month 7-24		
	Un-adjusted ABR	**Adjusted ABR (95% CI)**	**Un-adjusted ABR**	**Adjusted ABR (95% CI)**	**Rate Ratio (95% CI) p-value**	**Un-adjusted ABR**	**Adjusted ABR (95% CI)**	**Rate Ratio (95% CI) p-value**
Race								
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
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

Reviewer's Note:

The applicant interpreted the logarithm of uncontaminated time at risk of having a bleeding event was included as an offset variable. The unadjusted ABR, within a given time frame (eg, "Lead-in" or "Postdose Months 7 to 18"), was calculated as that ratio of total number of bleeding events and the total duration of uncontaminated time at risk over the entire full analysis set. The adjusted ABR, on the other hand, resulted from the negative binomial regression model. The disparity between the adjusted and unadjusted ABR in the specified subgroups was highly influenced by [redacted] *This subject experienced 5 total bleeds during Months 7 to 18 postdose but returned to routine prophylaxis within 5 weeks of Hemgenix administration, thus, having virtually zero "uncontaminated" time (0.003 years) to be considered at risk, resulting in extremely high ABR value. This outlying data point inflated the model-based results; however, it was absorbed within the unadjusted calculation.*

- ***Key secondary efficacy endpoints:***

Results of key secondary efficacy endpoints were provided in Table 6.3-5. As a result, using the pre-defined fixed-sequence approach, the first nine key secondary efficacy endpoints achieved statistical significance.

Table 6.3-5: Summary of Type I Error-Controlled Key Secondary Endpoints After 18 Months Post-treatment (Full Analysis Set)

	Secondary Efficacy Endpoint	Point Estimate	95% CI	One-sided p-value	Statistical Significance1
1.	Change From Baseline One-stage (aPTT*-based) FIX Activity (%) at 6 Months Post-treatment	36.00	31.47, 40.54	<0.0001	Yes
2.	Change From Baseline One-stage (aPTT-based) FIX (%) Activity at Year 1 Post-treatment	38.82	34.04,43.60	<0.0001	Yes
3.	Change From Baseline One-stage (aPTT-based) FIX (%) Activity at Month 18 Post-treatment	34.31	29.52,39.11	<0.0001	Yes
4.	Mean Difference in Annualized Consumption of FIX Replacement Therapy Use (IU/kg/yr; Month 7 to 18 Post-treatment – Lead-in Period)	-3056.8	-3642.8, -2470.8	<0.0001	Yes
5.	Adjusted Ratio for Annualized Infusion Rate of FIX Replacement Therapy (Month 7 to 18 Post-treatment: Lead-in Period)	0.03	0.01,0.10	<0.0001	Yes
6.	Odds Ratio One-stage (aPTT-based) FIX Activity <12% of Normal (Month 6 to 18 Post-treatment: Lead-in Period)	0.036	0.014,0.093	<0.0001	Yes
7.	Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period) for Superiority	0.36	0.20,0.64	0.0002	Yes
8.	Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period), Spontaneous Bleeding Episodes	0.29	0.12,0.71	0.0034	Yes
9.	Adjusted ABR Ratio (Month 7 to 18 Posttreatment: Lead-in Period), Joint Bleeding Episodes	0.22	0.10,0.46	<0.0001	Yes

10.	**LS Mean Difference in iPAQ Total Physical Activity Score (Post-treatment Period 1st Year–Lead-in Period)**	-721.2	-1770.6, 328.3	0.9121	No
11.	**LS Mean Difference in EQ-5D-5L VAS (Post-treatment Period 1st Year–Lead-in Period)**	0.1	-3.5,3.8	0.4753	No

*aPTT = activated Partial Thromboplastin Time

For the first three key secondary endpoints, change from baseline one-stage (aPTT*-based) FIX activity (%) at Month 6,12 and 18 Post-treatment, the applicant provided more detailed data as shown in Table 6.3-6.

Table 6.3-6: FIX Activity from One-stage (aPTT-based) Assay at 6 Months, 12 Months, and18 Months Post-AMT-061 Administration (Full Analysis Set)

	Result			**Change from Baseline**		
Visit	**n**	**Mean(SD)**	**Median(Min,Max)**	**LS Mean (SE)**	**95% CI**	**p-value**
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)	33.82(4.7,99.2)	34.13(2.325)	29.57,38.69	<0.0001

* "Uncontaminated" meant that the blood sampling did not occur within 5 half-lives of exogenous FIX use. 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. 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%.

Although FIX activity increased to clinically relevant levels following a single dose of 2×10^{13} gc/kg AMT-061 for the first three endpoints, we found that the LS means and 95% C.I.s in Table 6.3-6 were slightly different from the values in Table 6.3-5.

Reviewer's Note:

The applicant interpreted the differences in FIX activity results between the two tables as being due to different data cuts. Results in Table 6.3-5 were based on data cuts from Months 6 and 12, while those in Table 6.3-6 were based on data from Month 18. The model-based estimates changed due to the inclusion of additional data.

Reviewer's comments:

The efficacy results of Study CT-AMT-061-02 provided sufficient statistical evidence to support the non-inferiority of AMT-061 treatment to standard of care routine prophylaxis in terms of ABR for the efficacy evaluation period of Months 7 to 18 after AMT-061 treatment. In addition, the superiority of AMT-061 over standard of care was achieved.

6.4 External Communication (deficiency/inquiry and supplement, expert consultation…etc.)

The applicant's responses to the supplementary information have been included in the relevant chapters of the report.

6.5 Conclusion and Recommendation

In this submission, a Phase III clinical study, CT-AMT-061-02, has been provided to support the immunogenicity and efficacy of AMT-061. CT-AMT-061-02 is an open-label, single-dose, multi-center, multi-national trial, with a screening period, a lead-in phase, a treatment plus a post-treatment follow-up phase, and a long-term follow-up phase. Results of the primary and key secondary endpoints were summarized in Table 6.5. As a result, using the pre-defined fixed-sequence approach, the primary efficacy endpoint and the first nine secondary efficacy endpoints achieved statistical significance.

Table 6.5: Summary of Type I Error-Controlled Primary and Key Secondary Endpoints After 18 Months Post-treatment (Full Analysis Set)

Primary Efficacy Endpoint		**Point Estimate**	**95% CI**	**N.I. margin**	**Statistical Significance**
Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period) for Non-inferiority		0.36	0.20, 0.64	1.8	Yes
Secondary Efficacy Endpoint		**Point Estimate**	**95% CI**	**One-sided p-value**	**Statistical Significance**
1.	**Change From Baseline One-stage (aPTT*-based) FIX Activity (%) at 6 Months Post-treatment**	36.00	31.47, 40.54	<0.0001	Yes
2.	**Change From Baseline One-stage (aPTT-based) FIX (%) Activity at Year 1 Post-treatment**	38.82	34.04,43.60	<0.0001	Yes
3.	**Change From Baseline One-stage (aPTT-based) FIX (%) Activity at Month 18 Post-treatment**	34.31	29.52,39.11	<0.0001	Yes
4.	**Mean Difference in Annualized Consumption of FIX Replacement Therapy Use (IU/kg/yr; Month 7 to 18 Post-treatment – Lead-in Period)**	-3056.8	-3642.8, -2470.8	<0.0001	Yes
5.	**Adjusted Ratio for Annualized Infusion Rate of FIX Replacement Therapy (Month 7 to 18 Post-treatment: Lead-in Period)**	0.03	0.01,0.10	<0.0001	Yes
6.	**Odds Ratio One-stage (aPTT-based) FIX Activity <12% of Normal (Month 6 to 18 Post-treatment: Lead-in Period)**	0.036	0.014,0.093	<0.0001	Yes
7.	**Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period) for Superiority**	0.36	0.20,0.64	0.0002	Yes
8.	**Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period), Spontaneous Bleeding Episodes**	0.29	0.12,0.71	0.0034	Yes
9.	**Adjusted ABR Ratio (Month 7 to 18 Posttreatment: Lead-in Period), Joint Bleeding Episodes**	0.22	0.10,0.46	<0.0001	Yes
10.	**LS Mean Difference in iPAQ Total Physical Activity Score (Post-treatment Period 1st Year–Lead-in Period)**	-721.2	-1770.6, 328.3	0.9121	No
11.	**LS Mean Difference in EQ-5D-5L VAS (Post-treatment**	0.1	-3.5,3.8	0.4753	No

	Period 1st Year–Lead-in Period)				

*aPTT = activated Partial Thromboplastin Time

7. Clinical Evaluation

7.1 Introduction (product/disease background…etc.)

Product Information

This is the NDA application of Hemgenix® (Etranacogene dezaparvovec)1 × 10^{13} genome copies/mL, concentrate for solution for infusion by 傑特貝林有限公司.
Etranacogene dezaparvovec (**AMT-061**; AAV5-hFIXco-Pauda) is a gene therapy medicinal product that employs a recombinant adeno-associated viral vector serotype 5 (AAV5) containing a codon-optimized coding DNA sequence for the human coagulation Factor IX variant R338L (hFIXco-Padua) under the control of a liver-specific promoter (LP1). Following a single intravenous administration, Etranacogene dezaparvovec preferentially targets liver cells for transduction and resulting expression of FIX-Pauda protein at levels which modifies the severity of hemophilia B disease.
The applicant is seeking the following indication.

「Etranacogene dezaparvovec 為基因療法，以腺相關病毒為載體，已核准用於患有 B 型血友病 (先天缺乏第九凝血因子)，且既有 AAV5 中和抗體效價低於 1:900 的成人病人，以降低出血事件頻率，及滿足對第九凝血因子補充療法的需求，病人應：

- **目前使用第九凝血因子預防性療法，**
- **或目前或過去曾發生有生命危險的出血，或重複、嚴重自發性出血事件。」**

The proposed posology is single-dose intravenous infusion of 2 x 10^{13} gc/kg.

Disease Background

Hemophilia B is an X-linked inherited bleeding disorder characterized by an increased bleeding tendency due to either a partial or complete deficiency in the activity of the essential blood coagulation factor IX(FIX). Intra-articular and intramuscular bleeding are major clinical manifestations. Bleeding most commonly occurs in the knees, elbows, and ankles, etc. Recurrent bleeding can lead to hemophilic arthropathy, which further limits joint mobility, causes muscle atrophy and impairs function. The traditional classification of severity is based on residual FIX activity, severe <1%, moderate: ≥1-≤5%, mild: > 5% - < 40%. (1%=1 IU/dL, normal 50~150), each representing approximately 1/3 of the patients, respectively. Patients in the severe bleeding phenotype may be at risk of intracranial hemorrhage or even death if not treated prophylactically.
There is currently no treatment that can completely cure hemophilia B. A goal of hemophilia management is to prevent spontaneous bleeding events with replacement factor that increases and maintains FIX activity levels at moderate or even mild disease. Due to different injection frequencies (depending on the pharmacokinetic and pharmacodynamic nature of the product), differences in bleeding phenotypes, and economic and national drug supply issues, prophylactic treatment is currently recommended to adjust the dose/frequency based on individual bleeding status rather than using the same trough FIX level as a target for each patient.
The development of neutralizing antibodies, also known as inhibitors, in hemophilia is the most serious complication of factor replacement therapy. The development of an inhibitor in HB is a rare event (1.5-3% of all patients), but is associated with significant morbidity related not only to the risk of bleeding, but also to the frequent occurrence of allergic/anaphylactic reactions and nephrotic syndrome.
Currently, prophylactic treatment of hemophilia B can be performed mainly with intravenous injections of the recombinant product standard half-life (SHL) FIX or the

extended half-life (EHL)FIX. However, depending on the patient's bleeding condition and the selected medication, injections should be given every 2 weeks to a maximum of every 2-3 weeks. In Taiwan, there are several EHL products (e.g., Idelvion, Alprolix, Refixia., T1/2 ~ 86-104hr) approved for routine prophylaxis treatment of hemophilia B, the recommended injection interval (>12 years) are started with one week per injection, and up to 2 weeks interval according to individual bleeding condition. Children younger than 12 years old usually require more frequent injections to maintain the required FIX levels, due to the need for long-term and the prophylactic treatment often started since childhood, injection-related infections, blood clots, pain and burden is large for them.
Hemgenix is approved for the treatment of hemophilia B in several countries including the US and EU. The approved indication by USFDA and EMA is shown in the table below.
Table 1. The approved indication of US and EU

	Indication
US (2022/11/22)	HEMGENIX is an adeno-associated virus vector-based gene therapy indicated for the treatment of adults with Hemophilia B (congenital Factor IX deficiency) who: **• Currently use Factor IX prophylaxis therapy, or** **• Have current or historical life-threatening hemorrhage, or** **• Have repeated, serious spontaneous bleeding episodes.**
EU (2023/2/20)	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.**

7.2 Study designs

Source of Clinical Data/Tables of Clinical Trials

The main evidence for clinical efficacy and safety to support the proposed indication were pivotal trial **CT-AMT-061-02**(HOPE-B, N=54) and supportive trial CT-AMT-061-01 (N=3). Efficacy review focused on results of pivotal study, and safety database was comprised from two studies above.
Table 2. Overviews of Studies CT-AMT-061-01 and CT-AMT-061-02

Study	Design/No.	Study Design	Key Endpoint(s)
CT-AMT-061-**02(HOPE-B)** /US, EU, UK Pivotal, ongoing (2-year CSR & 3-year summary report)	Phase 3, OL, single-arm, **N=54**	Inclusion: under prophylaxis FIX treatment, severe or moderate severe**(FIX<2%)** Hemophilia B. **Including baseline AAV5 NAb positive.** – **Lead-in Period (26 weeks, Month 1-6):** routine prophylaxis FIX replacement therapy – **Dosing Visit**: $2x10^{13}$ gc/kg AMT-061 – **Post-treatment Follow-Up Period (52 Weeks)** – Long-term Follow-up Period (up to Month 60)	***Primary*** **ABR** (comparison between AMT-061 and prophylaxis between the lead-in phase and the post-treatment period) *Secondary* **FIX activity levels**, FIX replacement therapy consumption, QoL, HJHS, etc.

CT-AMT-061-01 /US Completed. (2018/7/24-2023/9/21)	Phase 2b, OL, single-arm **N=3**	Dosing visit: $2x10^{13}$gc/kg AMT-061 1-year post-treatment follow-up and 4 years of LTFU.	**FIX activity level**, FIX protein levels, FIX replacement therapy, bleeding episodes, PRO, etc.

ABR: annualized bleeding rate. OL: open-label. NAb: neutralized antibody. LTFU: Long-term follow-up

The sponsor initially submitted interim CSRs from two studies with a 2-year follow-up for the pivotal study (data cutoff: February 28, 2022) and a 3-year follow-up (data cutoff: December 14, 2021) of the CT-AMT-061-01 study. In response to CDE's clinical IR, the sponsor also provided a 3-year summary report for the pivotal study (CT-AMT-061-02) and a 5-year final CSR for the supportive study (CT-AMT-061-01).

[Reviewer's Note]: The CT-AMT-061-02, 3-year *summary report* contains only incomplete data, so only FIX information and partial safety data are used for this review. The rest of the information comes mainly from the full 2-year trial report.

CT-AMT-061-01, Dose-response study

Study Design and Summary of Results

CT-AMT-061-01 is a completed Phase IIb, single-arm study to confirm the endogenous Factor IX activity of Hemgenix administrated adult subjects with severe or moderate severe hemophilia B. A total 3 subjects were screened and treated in the study, all completed 5 years of follow-up. Subjects were all male, were 43, 47, and 50 years old, and two subjects were severe Hemophilia B, and one from moderately severe. They had baseline 1, 3, or 5 bleeding episodes in the year before screening, which were all spontaneous. Primary efficacy endpoint was FIX activity level at 6 weeks after dosing, mean (SD) uncontaminated FIX activity level was 30.6 (6.97). The increases in FIX activity were maintained through to Month 60 in these 3 subjects. (Table below)

Table 3. Uncontaminated Factor IX activity (%) from one-stage(aPTT-based) assay by visit

	[redacted]	n	Mean (SD)[1]
Baseline[2]		1	5.10
Week 3		3	23.40 (1.04)
Week 6		3	30.57 (6.97)
Week 52		3	40.77 (9.45)
Month 18		2	46.95 (12.66)
Month 24		3	44.20 (7.66)
Month 30		3	50.03 (11.40)
Month 36		2	36.90 (6.51)
Month 42		2	39.60 (5.80)
Month 48		3	45.00 (2.76)
Month 54		3	42.77 (7.17)
Month 60		3	45.67 (6.18)

Abbreviations: aPTT = activated partial thromboplastin time, FIX = factor IX, SD = Standard deviation.
"Uncontaminated" meant that the blood sampling did not occur within 5 half-lives of exogenous FIX use.
"Contaminated" results were defined to be a value from a blood sample obtained within 5 half-lives of previous FIX therapy. Both the data and time of exogenous FIX use (start) and the blood sampling were considered in determining contamination.

1. Contaminated values were not summarized and were excluded from analyses including the primary efficacy analysis unless otherwise specified.
2. Baseline = last available assessment prior to CSL222 administration.

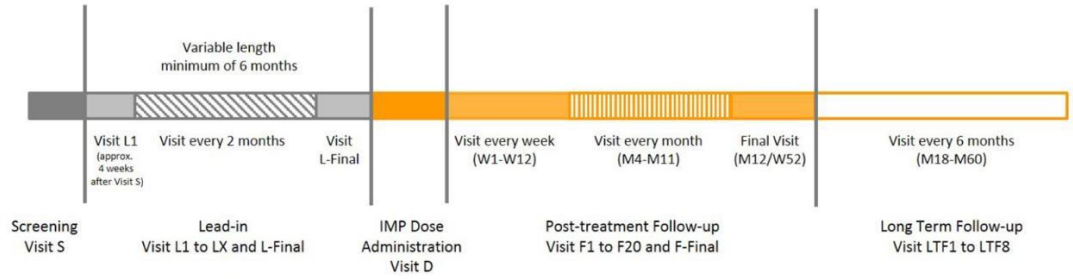
Pivotal Study, CT-AMT-061-02(HOPE-B)

Study Design

CT-AMT-061-02 is an ongoing open-label, single-dose, multi-center, multi-national trial, with a screening period, a lead-in period, a treatment plus a post-treatment follow-up period, and a long-term follow-up (LTFU) period.

During the lead-in period, which lasted a minimum of 26 weeks (i.e., ≥6 months), subjects recorded their use of FIX replacement therapy and bleeding episodes. After the lead-in phase, subjects received a single-dose of AMT-061(etranacogene dezaparvovec) and were followed for 1 year (i.e., post-treatment follow-up phase; 52 weeks) to evaluate efficacy and safety. Following the post-treatment follow-up phase, subjects continued into the long-term follow-up period for an additional 4 years.

Figure 1. Study Design



Abbreviations: IMP = Investigational Medicinal Product

Figure 2 Study Design

The primary efficacy endpoint is as follows:

1. Annualized bleeding rate (ABR) comparison between AMT-061 and prophylaxis for non-inferiority between the lead-in phase and the 52 weeks following stable factor IX expression. (Month 7-18 post-treatment)
    - Non-inferiority of AMT-061 was declared if the upper limit of the 97.5% CI of rate ratio in ABR between AMT-061 post-treatment and lead-in was less than the non-inferiority margin of 1.8.

The secondary efficacy endpoints were as follows:

2. Endogenous FIX activity at 6 months after AMT-061 dosing
3. Endogenous FIX activity at 12 months after AMT-061 dosing
4. Endogenous FIX activity at 18 months after AMT-061 dosing
5. Annualized consumption of FIX replacement therapy during the 52 weeks following stable FIX expression post-treatment follow-up, excluding FIX replacement for invasive procedures, compared to the lead-in phase
6. Annualized infusion rate of FIX replacement therapy during the 52 weeks following stable FIX expression post-treatment follow-up, excluding FIX replacement for invasive procedures, compared to the lead-in phase
7. 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
8. ABR comparison between AMT-061 and prophylaxis for superiority between the lead-in phase and the 52 weeks following stable FIX expression post-treatment
9. Rate of spontaneous bleeding episodes during the 52 weeks following stable FIX expression compared to lead-in phase

10. Rate of joint bleeding episodes during the 52 weeks following stable FIX expression compared to the lead-in phase
11. 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
12. PRO questionnaire scores from the EQ-5D-5L VAS score during the 12 months following AMT-061 dosing compared with the lead-in phase

Fixed sequential testing will be performed using a hierarchical approach and the order is specified above.

- Proportion of subjects remaining free of previous continuous routine prophylaxis during the 52 weeks following stable FIX expression
- Estimated ABR – during the 52 weeks following stable FIX expression– as a function of pre-IMP anti-AAV5 antibody titers using the luciferase based NAb assay
- Correlation of FIX activity levels during the 52 weeks following stable FIX expression with pre-IMP anti-AAV5 antibody titers using the luciferase based NAb assay
- Occurrence of (and resolution of) new target joints during the 52 weeks following stable FIX expression 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

The exploratory endpoints were as follows:

[redacted]

[Reviewer's Comment]: The primary efficacy endpoint of ABR is appropriate and clinically relevant. The study design adheres to the recommendations set forth by the USFDA guidance for hemophilia gene therapy, specifically with regard to intra-subject ABR comparisons. It also resembles the design of recently approved hemophilia products in Taiwan. *(USFDA Jan. 2020. Human Gene Therapy for Hemophilia Guidance for Industry)*

Main Inclusion Criteria

1. Male, age ≥18 years, with congenital hemophilia B with known severe or moderately severe FIX deficiency (≤2% of normal circulating FIX) for which the subject was on continuous routine FIX prophylaxis
2. >150 previous exposure days of treatment with factor IX protein
3. Have been on stable prophylaxis for at least 2 months prior to screening

Main Exclusion Criteria

1. History of factor IX inhibitors or positive factor IX inhibitor test at screening and Visit L-Final
2. Screening and Visit L-Final laboratory values: a. ALT >2 times upper normal limit (i.e., upper limit of normal; ULN) b. AST >2 times ULN c. Total bilirubin >2 times ULN (except if caused by Gilbert disease) d. Alkaline phosphatase (ALP) >2 times ULN e. Creatinine >2 times ULN
3. Positive human immunodeficiency virus (HIV) serological test at screening and Visit LFinal, not controlled with anti-viral therapy as shown by CD4+ counts ≤200/μL
4. 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).
5. Known coagulation disorder other than hemophilia B
6. Thrombocytopenia, defined as a platelet count below 50×10^9/L, at screening and Visit L-Final
7. Known significant medical condition that may significantly impact the intended transduction of the vector and/or expression and activity of the protein, including but not limited to: a. Disseminated intravascular coagulation b. Accelerated fibrinolysis c. Advanced liver fibrosis
8. Previous gene therapy treatment

7.3 Efficacy Results

Subject Disposition

A total of 75 subjects were screened and 67/75 (89.3%) subjects entered the lead-in period. Of the subjects who entered the lead-in period, 13/67 (19.4%) discontinued prior to dosing. There were 54/67 (80.6%) subjects treated with AMT-061, of which 53/54 (98.1%) subjects completed treatment. One subject discontinued the infusion prematurely due to hypersensitivity and received a partial dose (1/10 planned dose),

and this subject was not included in the PP population but in FAS. (PP set N=53, FAS N=54) The subject continued in the study for follow-up.
The majority of protocol deviations were related to timing of study visits or questionnaire completion, and the impact on the efficacy analysis is considered to be small.
[Reviewer's Note]: The subject received only 10% dose was reported 2 spontaneous bleeds and back to routine prophylaxis FIX replacement, so considered treatment failure.

Demographics and Baseline Characteristics

All subjects were male, the majority were White (74.6%), mean (SD) age was 41.5(15.8). Subjects presented with a mean (SD) hemophilia B duration of 39.7 (15.0) years. At the time of their diagnosis, 44/54 (81.5%) subjects had severe hemophilia B and 10/54 (18.5%) subjects had moderately severe hemophilia B. At screening, 3/54 (5.6%) subjects were HIV positive. There were 9/54 (16.7%) and 31/54 (57.4%) subjects with a history of hepatitis B and C, respectively. At screening, 13/67 subjects had target joints, and at dosing, 2/54 subjects had target joints.

Table 4. Summary of medical history related to hemophilia B(Safety Population)

Characteristic	Lead-in Safety Population Incl. Lead-in Discontinuers (N = 67)	Post-treatment Safety Population/FAS (N = 54)	PP Population (N = 53)
Severity of Hemophilia B at Time of Diagnosis, n (%)			
Severe[2]	56 (83.6)	44 (81.5)	43 (81.1)
Moderately Severe FIX[3]	11 (16.4)	10 (18.5)	10 (18.9)
FIX Replacement Therapy Type, n (%)[5]			
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)

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[4]	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)

Overall, 21 subjects had pre-existing NAbs against AAV5 at baseline (i.e., pre-dose), prior to AMT-061 treatment. The anti-AAV5 NAb titers 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**.

[Reviewer's Comment]: In response to the CDE's clinical information request, the sponsor provided an analysis of compliance with the prophylaxis regimen during the lead-in period. Compliance was calculated as the actual number of days the subject received the prophylactic FIX infusion, excluding the use of FIX for other purposes, divided by the total number of days the subject was to receive the prophylactic FIX as prescribed. During the prophylaxis lead-in period, 45 (83.3%) and 41 (75.9%) of 54 subjects were ≥ 70% and ≥ 80% compliant with the prophylaxis regimen, respectively. (Source: Response Table 67.2) The EHL product was used in approximately 57% of subjects. Based on the study design, baseline characteristics, and compliance with prophylactic rFIX during the lead-in period, the lead-in period may generally be representative of the real-world severe HB population with prophylactic rFIX.

Analysis of Endpoints

Primary Endpoint---ABR

The mean 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%; p = 0.0002]). The adjusted ABR rate ratio for the Month 7 to 18 post-treatment period to lead-in 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.

Table 5. Summary of Bleeding Episodes and Annualized Bleeding Rates (FAS)

	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, 2.76)	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

According to above result, superiority of Hemgenix compared to lead-in Period (secondary endpoint) was also demonstrated.

Key Secondary Endpoints

FIX activity

FIX activity significant increased and clinically relevant at Month 6, 12, 18, and were steady at Month 24 post-AMT-061 administration. No subject recorded values >150%.

Table 6. FIX Activity from One-stage (aPTT-based) Assay at 6 Months, 12 Months, 18 Months, and 24 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

There were 2 subjects whose FIX activity remained <2 or ≤5% after Hemgenix treatment and these 2 were non-responders (not expressing endogenous human FIX Padua protein after administration): One subject (FIX 1.5% at Week 5) with a high baseline NAb titer of 1:3212, another subject (FIX 1.7% at Week 11) who received a partial dose (~10% of the planned dose) due to hypersensitivity. All other subjects expressed endogenous FIX protein and had FIX>5%(OSA). (Source: response 3 iii)

[Reviewer's Comment]:

1. In response to CDE's clinical IR, the applicant submitted updated HOPE-B study 3-year summary report. There was steady mean FIX level until Month 36. (As below)

Visit	Result			Change from Baseline		
	N	Mean (SD)	Median (Min, Max)	LS Mean (SE) [a]	95% CI	p-value [b]
Month 36	47	38.63 (18.01)	35.60 (4.8, 80.3)	36.30 (2.265)	31.85, 40.74	< 0.0001

Currently, the applicant has provided 5-year results for 3 subjects (CT-AMT-061-01 study) and 3-year (HOPE-B) FIX results for 47 subjects. One subject loss of FIX expression for unknown reason after stable FIX expression for 2 years, but only 1 case (1.7%) are inconclusive. (Refer to safety review section AESI below for details). The overall FIX does not appear to be decreasing over time. However, durability results favored be at least 5-10 years to fully confirm long-term efficacy of FIX expression. The applicant explained that in addition to the HOPE-B 5-year follow-up, there is also a 15-year long-term observational trial, CSL222_4001, to follow up on the long-term safety and efficacy of gene therapy. After completing the reports of the two trials, the applicant is required to submit and update the package insert according to the long-term efficacy and safety result.

2. The one-stage aPTT-based assay (OSA) was used for FIX activity, and the blood-sampling did not occur within 5 half-lives of exogenous FIX use (defined as "uncontaminated" FIX activity in CSR). Such sampling methods are common and reasonable. However, the FIX activity measured by chromogenic assay was consistently approximately 2-fold lower than those by OSA (Refer to PK review report for details). Due to the different FIX assay used by medical institutions in Taiwan, the applicant should list the difference between the results of the chromogenic assay and OSA method in the package insert for the user's reference.

Exogenous FIX Replacement therapy

Consumption and infusions of FIX replacement therapy was significantly lower following treatment with AMT-061.

Table 7. Annualized Consumption of FIX Replacement therapy (IU/year, FAS)

	≥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]
Annualized Exogenous FIX Consumption (IU/year), n	54	54	54	54	53
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)	9750.8 (29,140.4)
Min; Max	83,541; 755,892	0; 204,899	0; 156,536	0; 180,618	0; 155,680

Table 8. Annualized Use of FIX Replacement Therapy(Infusions/year, FAS)

	≥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

Proportion of Subjects Remaining Free of Previous Continuous Routine Prophylaxis

All subjects received routine FIX prophylaxis during the ≥6-month lead-in period. Following treatment with Hemgenix, 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 baseline anti-AAV5 NAb titer (1:3213) [redacted]

Percentage of Subjects with Trough FIX Activity<12%

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(4/50 [8.0] %). 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.

ABR by Bleeding Subtype

As Table below, During the Month 7-18 post-treatment period, Spontaneous bleeding episodes and Joint Bleeding episodes were significant improvement with 71% and 78% reduction compared to lead-in period. These were considered clinically meaningful improvement over routine rFIX prophylaxis, because many of these bleeds in joints were progressed over time under prophylaxis.

Table 9. ABR Comparison by Bleeding Subtype

All Subjects (N = 54)	≥6-month Lead-in	Month 7-18			Month 7-24		
	ABR (95% CI)[1]	ABR (95% CI)[1]	Rate Ratio (Two-sided 95% Wald CI)[1]	p-value[2]	ABR (95% CI)[1]	Rate Ratio (Two-sided 95% Wald CI)[1]	p-value[2,3]
Spontaneous Bleeding Episodes	1.52 (1.01, 2.30)	0.44 (0.17, 1.12)	0.29 (0.12, 0.71)	0.0034	0.38 (0.16, 0.89)	0.25 (0.11, 0.57)	0.0005
Spontaneous FIX-treated Bleeding Episodes	1.34 (0.87, 2.06)	0.45 (0.15, 1.39)	0.34 (0.11, 1.00)	0.0254[3]	0.42 (0.15, 1.19)	0.31 (0.11, 0.87)	0.0127
Joint Bleeding Episodes	2.35 (1.74, 3.16)	0.51 (0.23, 1.12)	0.22 (0.10, 0.46)	<0.0001	0.46 (0.24, 0.89)	0.20 (0.10, 0.37)	<0.0001
Joint FIX-treated Bleeding Episodes	2.13 (1.58, 2.88)	0.44 (0.19, 1.00)	0.20 (0.09, 0.45)	<0.0001[3]	0.40 (0.20, 0.83)	0.19 (0.09, 0.38)	<0.0001
Traumatic Bleeding Episodes	2.09 (1.42, 3.08)	0.62 (0.31, 1.23)	0.30 (0.17, 0.52)	<0.0001[3]	0.58 (0.31, 1.09)	0.28 (0.17, 0.46)	<0.0001
Traumatic FIX-treated Bleeding Episodes	1.74 (1.21, 2.49)	0.22 (0.11, 0.45)	0.13 (0.06, 0.26)	<0.0001[3]	0.23 (0.13, 0.41)	0.13 (0.07, 0.25)	<0.0001
New and True Bleeding Episodes	3.83 (2.93, 5.01)	1.04 (0.52, 2.09)	0.27 (0.14, 0.52)	<0.0001[3]	1.07 (0.57, 2.00)	0.28 (0.16, 0.50)	<0.0001
New and True FIX-treated Bleeding Episodes	3.35 (2.57, 4.37)	0.64 (0.28, 1.43)	0.19 (0.09, 0.41)	<0.0001[3]	0.70 (0.35, 1.42)	0.21 (0.11, 0.42)	<0.0001

Summary of Key Secondary Endpoints

The other key secondary endpoints result summary is shown in the table below. The last two PRO endpoints (iPAQ, EQ-5D-5L) did not observed notable difference between the lead-in and post-treatment 1st year period. EQ-5D-5L VAS score observed improvement in the second-year post-treatment of 2.8 (1.40; 95% CI: 0.0, 5.6; p = 0.0244 [not adjusted for multiplicity]).

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. 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 VAS, which reflects the patient's perception of their overall health.

Table 10. Summary of Type I Error-Controlled Primary and Secondary Endpoints (FAS)

Endpoint	Point Estimate	95% CI	One-sided p-value	Statistical Significance[1]
Primary Efficacy				
Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period) for Non-inferiority[2]	0.36	0.20, 0.64	NA	Yes
Secondary Efficacy				
Change From Baseline One-stage (aPTT-based) FIX Activity (%) at 6 Months Post-treatment	36.00	31.47, 40.54	<0.0001	Yes
Change From Baseline One-stage (aPTT-based) FIX (%) Activity at Year 1 Post-treatment	38.82	34.04, 43.60	<0.0001	Yes
Change From Baseline One-stage (aPTT-based) FIX (%) Activity at Month 18 Post-treatment	34.31	29.52, 39.11	<0.0001	Yes
Mean Difference in Annualized Consumption of FIX Replacement Therapy Use (IU/kg/yr; Month 7 to 18 Post-treatment – Lead-in Period)	-3056.8	-3642.8, -2470.8	<0.0001	Yes
Adjusted Ratio for Annualized Infusion Rate of FIX Replacement Therapy (Month 7 to 18 Post-treatment: Lead-in Period)	0.03	0.01, 0.10	<0.0001	Yes
Odds Ratio One-stage (aPTT-based) FIX Activity <12% of Normal (Month 6 to 18 Post-treatment: Lead-in Period)	0.036	0.014, 0.093	<0.0001	Yes
Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period) for Superiority	0.36	0.20, 0.64	0.0002	Yes
Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period), Spontaneous Bleeding Episodes	0.29	0.12, 0.71	0.0034	Yes
Adjusted ABR Ratio (Month 7 to 18 Post-treatment: Lead-in Period), Joint Bleeding Episodes	0.22	0.10, 0.46	<0.0001	Yes
LS Mean Difference in iPAQ Total Physical Activity Score (Post-treatment Period 1st Year – Lead-in Period)	-721.2	-1770.6, 328.3	0.9121	No
LS Mean Difference in EQ-5D-5L VAS (Post-treatment Period 1st Year – Lead-in Period)	0.1	-3.5, 3.8	0.4753	No

[Reviewer's Comment]: In the adult hemophilia B population, physical activity and quality of life are likely to be primarily composed of hemophilic arthropathy and the burden of frequent injections. Under the circumstances that most subjects received prophylaxis with EHL product, adults with hemophilic arthropathy at this age may be less likely to produce very noticeable symptoms or functional improvement within one year.

Other Endpoints with Clinical Special Meaning

Subjects with Zero Bleeding Episodes

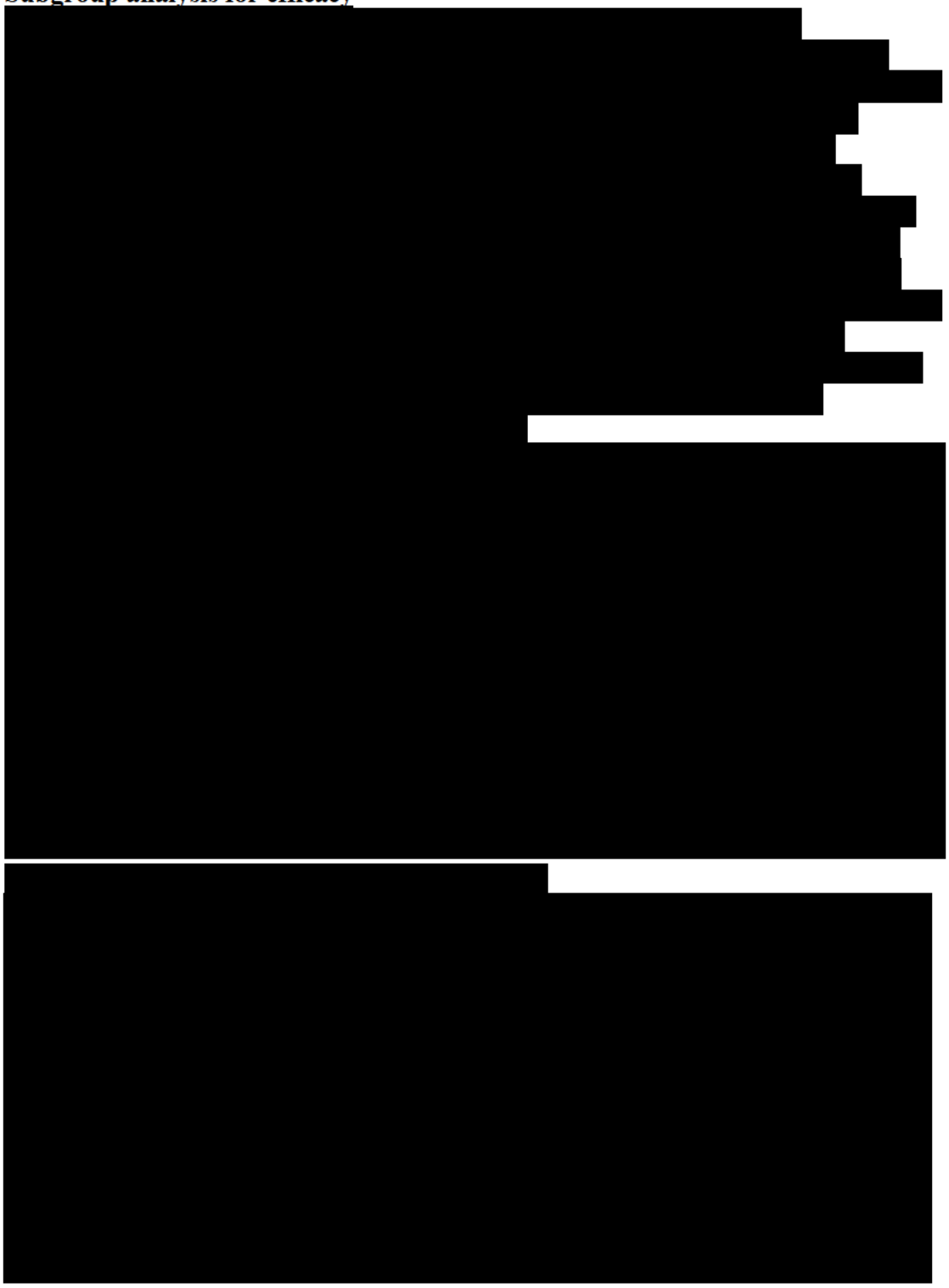
The number (%) of subjects with zero bleeding episodes increased following treatment with AMT-061, from 14/54 (25.9%) subjects during the ≥6-month lead-in period, to 34/54 (63.0%) subjects during the Month 7 to 18 post-treatment period.

Rate of Traumatic Bleeding Episodes

During the ≥6-month 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 9/54 (16.7%) subjects during the Month 7 to 12 period and 14 episodes in 7/54 (13.0%)

subjects during the Month 13 to 18 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, and 0.58 (95% CI: 0.31, 1.09) for the Month 7 to 24 post-treatment period.

Subgroup analysis for efficacy



[redacted]

FIX activity and Baseline Anti-AAV5 NAb

Hemgenix is AAV vector-based gene therapy, pre-existing anti-AAV NAbs may impede FIX expression. In HOPE-B study, NAbs were present in 21/54 (38.9%) subjects at baseline. 20/54 (37.0%) subjects had values up to 1:700 (range: 8.5 to 678.2), and 1 subject had high titer to 1:3212.3. Baseline mean FIX activity was similar (~1%) between subjects with and without anti-AAV5 NAb. In response to CDE's clinical IR, the applicant submitted the additional post-hoc subgroup analysis of FIX activity by negative anti-AAV5 NAb(N=33) and up to 1:700 positive anti-AAV5 NAb(N=20) as table below. There were numerical lower mean FIX level in patient with pre-existing NAbs(<1:700), though the mean FIX level still above 30% through post-treatment 24 Months.

Table 14. Uncontaminated Central Laboratory Data from One-Stage aPTT Assay for Factor IX Activity (%) by Visit in the Post-Treatment Period and Subcategory: Baseline Neutralizing Antibodies to AAV5 (Subjects with Baseline NAb titer < 3000)

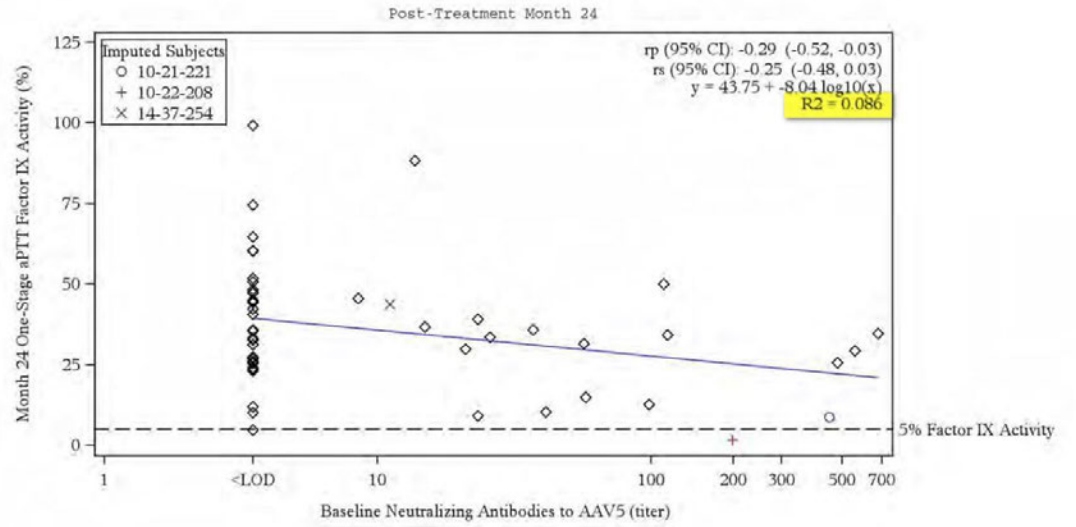
	Patients With* Pre-Existing Neutralizing Antibodies to AAV5 (N=20)		Patients Without* Pre-Existing Neutralizing Antibodies to AAV5 (N=33)	
	Result	Change From Baseline	Result	Change From Baseline
Baseline [a]				
n	20		33	
Mean (SD)	1.25 (0.44)		1.15 (0.36)	
Q1	1.00		1.00	
Median	1.00		1.00	
Q3	1.50		1.00	
Minimum, Maximum	1.0, 2.0		1.0, 2.0	

Post-Treatment Month 12				
n	18	18	32	32
Mean (SD)	35.54 (17.84)	34.32 (17.86)	44.82 (23.21)	43.70 (23.28)
Q1	16.30	15.30	31.15	29.65
Median	39.95	38.95	38.65	37.65
Q3	47.90	46.90	56.20	55.20
Minimum, Maximum	8.5, 73.6	6.5, 72.6	5.9, 113.0	4.9, 112.0
LS Mean [b] (SE)		32.57 (3.921)		43.10 (2.937)
95% CI		(24.86, 40.28)		(37.33, 48.87)
One-sided P-Value [c]		<0.0001		<0.0001
Post-Treatment Month 18				
n	17	17	33	33
Mean (SD)	31.14 (13.75)	29.90 (13.74)	39.87 (24.08)	38.72 (24.16)
Q1	21.60	19.60	25.60	24.60
Median	32.00	31.00	35.00	34.00
Q3	39.80	37.80	48.50	47.50
Minimum, Maximum	10.3, 57.9	9.3, 56.9	4.5, 122.9	3.5, 121.9
LS Mean [b] (SE)		27.64 (3.928)		38.72 (2.933)
95% CI		(19.91, 35.37)		(32.96, 44.48)
One-sided P-Value [c]		<0.0001		<0.0001
Post-Treatment Month 24				
n	17	17	33	33
Mean (SD)	32.98 (18.51)	31.75 (18.49)	38.55 (19.19)	37.40 (19.27)
Q1	25.60	23.60	26.10	25.10
Median	33.50	32.50	35.40	34.40
Q3	36.70	34.80	47.40	46.40
Minimum, Maximum	9.1, 88.3	8.1, 87.3	4.7, 99.2	3.7, 98.2
LS Mean [b] (SE)		29.33 (3.932)		37.40 (2.933)
95% CI		(21.59, 37.06)		(31.64, 43.16)
One-sided P-Value [c]		<0.0001		<0.0001

Source: response #5 ii, Table 2.1.2.3

The linear regression of subjects with baseline anti-AAV5 NAb <1:700 is shown in the figure below. The figure shows a trend toward lower mean FIX activity in subjects with anti-AAV5 NAbs at baseline. However, there was no clinically meaningful correlation between an individual's titer of pre-existing anti-AAV5 NAbs and their FIX activity at 18 months or 24 months post-treatment with low R^2(0.086).

Figure 4. Linear regression, FIX activity from One-stage aPTT-based assay at Month 24 post-treatment by Baseline anti-AAV5 Nab in Subjects with titer<700(FAS)



[Reviewer's Comment]:

The Applicant proposed "既有 AAV5 中和抗體效價低於 1:900 的成人病人" for included in the indication. Regarding the target population for this indication, the discussion is as follows:

1. In response to CDE's clinical IR, the applicant explains that *the selection of the 1:900 cutoff was derived from an analytical method comparison study that assessed serum samples from 30 healthy donors with the original clinical study AAV5 NAb assay (using 7 serum dilution steps) and the modified AAV5 NAb assay with expanded measuring range (using 9 serum dilution steps). This comparative analysis concluded that a titer of 1:678 in the original clinical study assay corresponded to approximately 1:900 in the modified assay with expanded measurement range.* [redacted]

[redacted] (Note: The applicant did not provide complete study reports related to the above assays. The new 9-point assay (PfM AAV5 NAb assay) is CE marked in the EU and bears United Kingdom (UK) Conformity Assessment (UKCA) Marking, according to response #5 iv). [redacted] But the sponsor has not reanalyzed the samples from the pivotal study using the new 9-point "validated" AAV5 NAb, so extrapolating clinical efficacy from the study population with the 7-point AAV5 NAb assay to the new 9-point assay is still full of uncertainty.

2. There is limited data analyzing the correlation between pre-treatment anti-AAV5 NAbs and post-treatment FIX levels. However, in the proposed population of less than 1:700 (original 7-point assay, approximating the new method of 1:900), clinically meaningful efficacy was observed in the pivotal study. However, one subject with a higher titer (1:3212) did not respond with post-treatment FIX expression, and based on the mechanism of the AAV vector, it is likely that the presence of anti-AAV NAbs prior to treatment would affect post-treatment FIX expression. The exact relevance and thresholds are unknown as data on 700-3200 titers are limited and the original assay is not validated. The applicant is conducting a study (CSL222_3005, initiated 2024/1) to assess whether there is a clinically meaningful correlation between pre-treatment AAV5 NAb titers and the risk of bleeding after Hemgenix treatment. Assuming that the <1:900 indication restriction is not maintained, this may result in some patients being ineffective after administration. Although clinicians can monitor bleeding risk by monitoring FIX and bleeding status, this group of patients may receive gene therapy without the possibility of benefit. [redacted] However, it would be too long to wait for the ongoing correlation trial to be completed, and the efficacy of the product in a subset of NAbs-positive patients has already been demonstrated in the current pivotal trial, although the threshold has not yet been confirmed. Therefore, the CDE recommends that the applicant's proposed <1:900 indication group restriction be maintained, that detailed information about the NAb should be provided in the package insert and RMP for healthcare providers and patients, and that the PI and/or the indication should be updated upon completion of the CSL222_3005 trial.

7.4 Safety Results

Assessment Methods and Extent of Exposure

Two clinical studies (AMT-061-02, N=54; AMT-061-01, N=3) provide safety data for Hemgenix in total 57 exposed adult subjects (ISS safety population). Pivotal study contributed the majority of the safety analysis. Most subjects (50) in pivotal study have been followed for at least 2 years post-treatment. Overall exposure was 1547.5 person-months (Table below).

Table 15. 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

AE profiles

Overview of Safety

The incidence and number of AEs is higher after treatment, compared to the lead-in period. Notably, there is longer follow-up period in Post-treatment period (most 24 months) compared to lead-in period (≥6 months). However, even if this factor is removed, the overall incidence of AE should still be significantly higher than the lead-in period with prophylaxic agent due to the large difference in values.

Table 16. Overview of AEs

	Lead-in Period (Including Lead-in Discontinuers) (N = 67)		Lead-in Period (Excluding Lead-in Discontinuers) (N = 54)		Post-treatment Period (N = 54)	
	n (%)	# of Events	n (%)	# of Events	n (%)	# of Events
At least one AE[1]	42 (62.7)	103	37 (68.5)	87	54 (100.0)	557
Mild AE	41 (61.2)	78	37 (68.5)	67	54 (100.0)	424
Moderate AE	12 (17.9)	21	9 (16.7)	17	37 (68.5)	115
Severe AE	3 (4.5)	4	2 (3.7)	3	11 (20.4)	18
AEs related to study treatment[2]	0		0		38 (70.4)	93
AEs of special notification	0		0		12 (22.2)	19
AEs leading to premature treatment discontinuation	0		0		1 (1.9)	1
Serious AEs	5 (7.5)	7	4 (7.4)	5	14 (25.9)	17
Serious AEs related to study treatment	0		0		0	
Deaths – all causes	0		0		1 (1.9)	1

Common AEs

During the Lead-in Period in Study CT-AMT-061-02, subjects received routine prophylaxis rFIX replacement therapy. Common AEs (> 5% of subjects) by Preferred Term (PT) during the Lead-in Period (excluding discontinuers; N = 54) were Nasopharyngitis (14.8%; 8 events) and Arthralgia (7.4%; 4 events). All other AEs occurred in 2 (3.7%) or fewer subjects. It appears to be related to the background population of hemophilia B. (i.e., hemophilic arthropathy)

In Post-treatment period, 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%).

Table 17. Incidence and Number of TEAEs by PT in ≥5% of Subjects (ISS 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

According to the applicant response to CDE's IR, the common treatment-*related* TEAEs were ALT Increased, Headache, Influenza-like Illness, AST Increased, blood CPK increased, nausea, fatigue. (Source: response #2 vi, appendix Justification for ADRs-CSI)
[Reviewer's Comment]: Given the characteristics of this AAV5-based gene therapy product, the symptoms associated with the background of hemophilia, and the temporal sequence and incidence difference in which TEAE occurs, it is agreed that the applicant's discussion of these PT is related to this product. However, the incidence values of certain items originally presented in the draft form differed from those analyzed by the CDE (e.g., elevated ALT, AST, influenza-like illness), and after revised, the current ADRs list was deemed to provide sufficient information for the prescriber to refer to.

Serious AEs

In the ISS Safety Population, 15 (26.3%) subjects experienced 18 treatment-emergent SAEs. Serious AEs with PT blood loss anemia were reported for 2 (3.5%) subjects; no other SAEs were reported in more than 1 subject. In two SAE events with blood loss anemia, one (#10-14-201, event on Study Day 999) occurred in subject who with rectal bleeding from hemorrhoids, and the other subject with anemia event (#10-22-208, subject with partial Hemgenix dose [~10%] and back to routine prophylaxis FIX replacement, event on Study Day 736) developed from diverticulitis hemorrhage. Two events were considered unrelated to Hemgenix.

Hepatocellular carcinoma (HCC)

A subject [redacted] with moderately severe HB, developed HCC on Study Day 365. He had relevant history of hepatitis B history (anti-HBc positive, HBs Antigen negative), hepatitis C (dx in 2003, HCV eradication from 2015/3-2016/6), alcohol use, and fatty liver, etc. Pre-treatment Fibroscan showed F0/F1(5.7kPa), liver function (PLT, albumin, bilirubin, PT) showed normal, and mild elevated AST/ALT(<2X) before treatment.

On Study Day 365, ultrasound per protocol found a subcapsular lesion and was subsequently confirmed as HCC. He received surgical excision on Study Day 443.

Results of the integration site (IS) analysis revealed

56 unique IS in the HCC and 39 unique IS in the HCC-adjacent sample respectively. These indicate that <0.03% of the cells in the HCC and HCC-adjacent tissues had AAV integration. A dominant IS was not identified, as would be expected had the AAV vector integrated and led to clonal expansion of the tumor cells. Whole genome sequencing (WGS) identified five additional integration sites 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, microRNA analysis identified genes known to be associated with the progression and development of HCC. The Sponsor 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. Four independent external experts reviewed this data and reached the same conclusion.

[Reviewer's Comment]: Although the possibility of accelerated HCC progression cannot be completely excluded, considering the various risk factors, the clinical course and the analysis result, the clinical reviewer agrees that the applicant does not attribute this case of HCC to Hemgenix. Although the frequency of random chromosomal integration is not high in non-clinical data, the theoretical risk of HCC development still exists. The potential carcinogenic risk still needs to be mentioned in the label warning, as longer-term safety data are still needed to fully confirm the associated risks.

Transient ischemic attack

One subject [redacted] with severe hemophilia B, experienced a transient ischemic attack on Study Day 229. He had several risks or relevant past history of HTN, CAD s/p bypass, aortic valve stenosis, aortic valve replacement, peripheral artery aneurysm, etc. And there was a previous TIA event in 2018/11

before treatment. The FIX activity was stable in range of 34-53% after stable FIX expression post-treatment. 9 days prior to TIA event, his FIX level was 40%. On Study Day 229(2020/9/26), the subject experienced TIA. The Investigator considered the event unlikely to be related to study medication.
[Reviewer's Comment]: The TIA event was likely unrelated to Hemgenix due to several significant risk factors.

Chest pain
A subject [redacted] with severe Hemophilia B, experienced a SAE of chest pain. On Post-treatment Study Day 2 (2019/10/23), the subject experienced mild pain in chest wall and was admitted to the hospital on Study Day 3 for evaluation. An ECG, echo were normal, and no signs of heart disease or pulmonary embolism were identified. Treatment of the event included nitroglycerin and amlodipine. Chest pain improved one day later, and recorded resolved and discharge on Study Day 4. The investigator presumed that this SAE may be related to exercise, and assessed not related to the study medication.
[Reviewer's Comment]: According to the clinical course, it can not be rule out the possible relation to Hemgenix, though it is mild in nature.
(SAE narratives, safety-data-and-figures 14.3.3.2)

Death
A subject [redacted] reported death in ISS safety population (Study CT-AMT-061-02). A [redacted] with severe hemophilia B, had history of atrial enlargement, atrial fibrillation, BPH, intestinal diverticulum, HCV, HTN, osteoporosis, experienced a fatal event of Cardiogenic Shock. On Study Day 461, the subject was diagnosed UTI and sepsis, and cefixime was prescribed. On Study Day 463, he experienced a SAE of cardiogenic shock. The subject presented to the emergency department with "heart-pounding" palpitations since earlier the same morning and a 3-day history of fever with dysuria. ECG found Afib with a mean of 110 bpm, CXR revealed congestion with blurred consolidation. On Study Day 464, the condition progressed with dyspnea and subsequent cardiorespiratory arrest. CPR was performed twice but still fatal. An autopsy was not performed. The Applicant considered not related to study medication.
[Reviewer's Comment]: Clinical reviewer consider the event is unlikely related to Hemegenix based on the multiple comorbidities of the subject.

Laboratory Findings
No clinically meaningful variations from baseline values were noted in the serum chemistry and hematology parameters after Hemgenix treatment, except elevated liver enzymes. For details on abnormal transaminase testing, see the AESI section below. No notable differences were observed in coagulation results.
There were 2 subjects with elevated Alpha-fetoprotein (AFP) post-treatment. One subject had high baseline AFP value and relative stable value through post-treatment month 24(baseline: 9.35 IU/mL, Month 6-24: 10.20-10.40 IU/mL, normal range: 0-6.9). One subject had one elevated AFP value at Month 12 visit. Overall, AFP levels appeared unaffected by Hemgenix.

AE of Special Interest
Abnormal Liver laboratory test and TEAEs of Transaminase elevations

Hemgenix is an AAV5-based gene therapies that targeted to the liver cells which may lead to transaminitis. Transaminases may be due to T-cell mediated responses, and since FIX is synthesized by hepatocytes, transaminases may also affect FIX expression and/or increase the risk of bleeding. The review related to transaminases is discussed as follows.

The mean change in laboratory ALT at post-treatment month 24 was slightly elevated, but the mean values remained within normal ranges (baseline mean [SD]: 20.8 U/L [12.5]; post-treatment month 24 change from baseline 4.5 U/L [17.7], CT-AMT-061-02 study) with fluctuating mean change from baseline through the different visits. 17 subjects (31.5%) had elevated ALT>2x baseline, and 9(16.7%) had elevated AST>2x baseline levels post Hemgenix treatment.

In response to CDE's clinical information request, the applicant provided new occurring or worsening hepatic lab abnormality, as below.

Table 18. New occurring or worsening hepatic laboratory abnormality.

	Number of Patients (%) (N=54)
ALT > ULN	16 (29.6)
>ULN - 2xULN	11 (20.4)
>2xULN - 3xULN	3 (5.6)
>3xULN - 5xULN	1 (1.9)
>5xULN	1 (1.9)
AST > ULN	13 (24.1)
>ULN - 2xULN	10 (18.5)
>2xULN - 3xULN	3 (5.6)
>3xULN - 5xULN	0
>5xULN	0
Bilirubin > ULN	14 (25.9)
>ULN - 2xULN	12 (22.2)
>2xULN - 3xULN	2 (3.7)
>3xULN - 5xULN	0
>5xULN	0

16 subjects who had elevated ALT>ULN values from Day 9 to 738 post administration. 10 subjects (62.5%) had elevated ALT levels within the first 3 months after administration, with 9 of them resolved within 3 months. One subject with ALT>5x ULN occurred 3 weeks (Day 24) after treatment and resolved on Day 65 after steroid treatment. None of these elevations were associated with a serious adverse event (SAE). (Source: Response # 2 ii, Listing 128.1)

9(15.8%) subjects received steroids as treatment for the liver enzyme elevations of either > ULN (n = 8) or > 2 × baseline value (n = 1). 5 of the subjects receiving steroid had an isolated ALT increase and 4 had both AST and AST evaluation.

All the 9 subjects had an onset within 3 months post-dose, with the earliest onset at Week 3. All subjects discontinued steroid use before Week 26. The mean corticosteroid treatment duration for those subjects was 79.8 days (range 51 to 130 days). At 24 months post-dose, all subjects who experienced a central laboratory-based ALT > ULN elevation and all subjects who were treated with corticosteroids for a TEAE of elevated ALT remained off continuous routine prophylaxis. No subject met the definition of drug-induced liver injury.

[Reviewer's Comment]: There were total 24 subjects (~42%) with elevated ALT if defined as ">ULN" OR ">2x baseline". This is a somewhat conservative standard, however, given the potential for a decline in the FIX as a result of a transaminase increase, and the fact that not all subjects recover their endogenous FIX expression when transaminase is restored. Furthermore, it cannot be confirmed whether relatively low FIX expression (e.g. <10%) affects long-term (e.g., 5+ years) efficacy persistence, so CDE suggests that a more conservative result be presented in the Labeling.

By TEAEs of transaminase elevations, there were 11/54 (20.4%) subjects with 12 TEAEs of ALT increased, 8/54 (14.8%) subjects with 9 TEAEs of AST increased, and 1/54 (1.9%) subject with 1 TEAE of transaminases increased. There were 4 subjects with anti-AAV5 NAb positive, 9 subjects with anti-AAV5 negative.
Table 19. Summary of Liver enzyme elevated AEs

Subject	Pre-dose anti-AAV5 NAb Titer	TEAE Preferred Term (Duration)	TEAE Severity	Relationship[1]	Elevation >2 × baseline	Treatment (Duration)
10-10-213	<LOD	ALT increased (Study Day 22 to 36)	Moderate	Related	Yes	Prednisolone (Study Day 22 to 85)
10-12-257	<LOD	AST increased (Study Day 22 to 29)	Mild	Related	No	None
10-16-238	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)
10-18-268	<LOD	ALT increased (Study Day 30 to 50)	Moderate	Related	Yes	Prednisolone (Study Day 36 to 38); Prednisone (Study Day 38 to 86)
10-19-214	<LOD	ALT increased (Study Day 36 to 149)	Mild	Not related	No	Prednisone (Study Day 49 to 149)
10-21-207	<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
10-25-203	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
10-61-269	<LOD	AST increased (Study Day 74 to 85)	Mild	Related	No	None
		ALT increased (Study Day 74 to 106)	Mild	Related	Yes	None
11-31-264	98.5	ALT increased (Study Day 35 to 42)	Moderate	Related	Yes	Methylprednisolone (Study Day 43 to 98)
13-34-234[4]	<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)
13-36-245	<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
16-43-242	<LOD	ALT increased (Study Day 59 to 71)	Mild	Related	No	Prednisolone (Study Day 61 to 134)
16-43-247	13.7	AST increased (Study Day 728 to 746)	Mild	Not related	Yes	None

The applicant submitted the new HOPE-B Listing 128.1 to show the laboratory liver enzyme > ULN result, there were 4 subjects with positive pre-existing anti-AAV5 NAb(titer 13.7-449.9).
Overall, there is no significant trend or correlation between elevated liver enzyme and pre-existing anti-AAV5 NAb.

The applicant submitted the new CT-AMT-061-02 post-hoc Table below to show the elevated ALT subgroup efficacy analysis.
Overall, with or without steroid treatment, mean FIX tended to be lower but still higher than 25%(OSA) in ALT >ULN subgroup, and ABR was similar between the ALT normal or the >ULN subgroups.
Table 20. One-stage aPTT uncontaminated FIX activity by ALT subgroups(FAS)

	No ALT Elevation (N=38)	>ULN - 2xULN (N=11)	>2xULN - 3xULN (N=3)	>3xULN - 5xULN (N=1)	>5xULN (N=1)	>ULN (Total) (N=16)
Baseline [a]						
n	38	11	3	1	1	16
Mean (SD)	1.16 (0.370)	1.36 (0.505)	1.00 (0.000)	1.00 (-)	1.00 (-)	1.25 (0.447)
Week 6						
n	33	10	3	1	0	14
Mean (SD)	35.10 (13.987)	26.14 (15.630)	18.63 (2.804)	45.30 (-)		25.90 (14.544)
Month 3						
n	35	11	3	1	1	16
Mean (SD)	39.60 (17.194)	32.72 (18.805)	18.60 (1.375)	64.20 (-)	10.10 (-)	30.63 (19.271)
Month 6						
n	36	10	3	1	1	15
Mean (SD)	41.78 (17.395)	36.61 (21.147)	16.13 (6.052)	54.90 (-)	13.00 (-)	32.16 (20.617)
Month 12						
n	35	10	3	1	1	15
Mean (SD)	44.36 (18.964)	38.64 (26.135)	15.00 (8.525)	77.30 (-)	12.80 (-)	34.77 (26.601)
Month 18						
n	35	10	3	1	1	15
Mean (SD)	40.55 (21.773)	32.66 (18.431)	12.90 (7.988)	48.50 (-)	12.00 (-)	28.39 (18.439)
Month 24						
n	35	10	3	1	1	15
Mean (SD)	39.59 (17.733)	34.72 (21.318)	14.20 (9.215)	47.20 (-)	10.30 (-)	29.82 (20.555)

Table 21. ABR by ALT subgroups(FAS)

Table 128.2 Annualized Bleeding Rates by ALT Elevation Subgroups (Full Analysis Set)

	No ALT Elevation (N=38)	>ULN - 2xULN (N=11)	>2xULN - 3xULN (N=3)	>3xULN - 5xULN (N=1)	>5xULN (N=1)	>ULN (Total) (N=16)
Lead-In						
Number of Bleeds	94	33	5	1	3	42
Number of Person-years Observed for Bleeding Events	23.59	6.36	1.97	0.60	0.59	9.52
Unadjusted Annualized Bleeding Rate [a]	3.98	5.19	2.53	1.68	5.07	4.41
Adjusted Annualized Bleeding Rate (95% CI) [b]	4.03 (2.96, 5.48)	5.27 (2.90, 9.55)	2.32 (0.69, 7.81)			4.55 (2.75, 7.50)
Month 7-24						
Number of Bleeds	34	30	5	2	3	40
Number of Person-years Observed for Bleeding Events	51.69	15.82	4.22	1.49	1.35	22.88
Unadjusted Annualized Bleeding Rate [a]	0.66	1.90	1.18	1.35	2.23	1.75
Adjusted Annualized Bleeding Rate (95% CI) [b]	1.49 (0.61, 3.65)	2.00 (0.83, 4.81)	1.34 (1.00, 1.79)			1.80 (0.92, 3.54)
Rate Ratio (95% CI) [b]	0.37 (0.16, 0.87)	0.38 (0.20, 0.73)	0.58 (0.13, 2.50)			0.40 (0.24, 0.66)
P-value [c]	0.0110	0.0018	0.2314			0.0002

Table 22. One-stage aPTT uncontaminated FIX activity by ALT elevation treated with corticosteroid subgroup

	ALT Elevation Treated with Corticosteroid (N=9)	Other (N=45)
Baseline [a]		
n	9	45
Mean (SD)	1.22 (0.441)	1.18 (0.387)
Week 6		
n	7	40
Mean (SD)	19.51 (8.980)	34.61 (14.323)
Month 3		
n	8	43
Mean (SD)	21.83 (11.768)	39.57 (17.871)
Month 6		
n	9	42
Mean (SD)	18.72 (11.086)	43.29 (17.155)
Month 12		
n	8	42
Mean (SD)	16.70 (9.696)	46.20 (20.118)
Month 18		
n	9	41
Mean (SD)	15.56 (7.920)	41.59 (20.573)
Month 24		
n	9	41
Mean (SD)	15.52 (7.710)	41.30 (17.486)

Table 23. ABR by ALT elevation treated with corticosteroid subgroup

Table 128.3 Annualized Bleeding Rates by Systemic Corticosteroid Exposure Subgroups (Full Analysis Set)

	ALT Elevation Treated with Corticosteroid (N=9)	Other (N=45)
Lead-In		
Number of Bleeds	20	116
Number of Person-years Observed for Bleeding Events	5.54	27.57
Unadjusted Annualized Bleeding Rate [a]	3.61	4.21
Adjusted Annualized Bleeding Rate (95% CI) [b]	3.71 (2.28, 6.05)	4.26 (3.16, 5.75)
Month 7-24		
Number of Bleeds	12	62
Number of Person-years Observed for Bleeding Events	12.84	61.72
Unadjusted Annualized Bleeding Rate [a]	0.93	1.00
Adjusted Annualized Bleeding Rate (95% CI) [b]	0.93 (0.47, 1.84)	1.96 (0.90, 4.25)
Rate Ratio (95% CI) [b]	0.25 (0.13, 0.50)	0.46 (0.22, 0.96)
P-value [c]	<0.0001	0.0191

Note: 1. According to protocol, for ALT level increments of at least 2-fold baseline (i.e., Visit D, pre-IMP) and/or > ULN, by local or central laboratories, the Investigator should Investigators should assess potential causes and discuss with Medical Directors, and/or initiation of corticosteroid treatment. In case of AST level increments > ULN, a similar process took place.
2. There were 3 of 9 subjects treated with corticosteroids for elevated ALT without exceed ULN in central laboratory data. 2 of them had a peak ALT>2 baseline, 1 subject had ALT>2X ULN only in local laboratory data with central lab not measured due to unscheduled visit.

In updated HOPE-B 3 year summary report, 1 subject [redacted] loss of his FIX expression between Month 29 and Month 30 post-treatment He demonstrated early liver transduction with FIX expression > 20% (Week 3-4), and experienced an asymptomatic episode of mild ALT increased (41, local lab ULN:40) at Week 5, along with decrease of FIX expression (Week 4 22.2% to Week 5 15.5%). The subject did well clinically with minimal bleeding and with FIX activity approximately 9% to 12% for 2 years. Between Months 29 and 30 after CSL222 treatment, he experienced an increased number of hemorrhages and was found to have a FIX activity (local and central laboratory) < 5%, and return to continuous prophylactic FIX infusions. *[Reviewers Note]:* This case is [redacted], baseline anti-AAV5 1:98.5. Although the applicant states that the timing of the slight increase in ALT was similar to the timing of the decrease in FIX, there was only a slight increase in ALT at week 5 (and this was a local lab result, central lab ALT was 35, within normal range), and all subsequent time points were within the normal range. In this subject, FIX increased briefly to 30.3% at week 9 and then stabilized in the 9-15% range for the next 2 years. As mentioned above, the reason for the loss of expression at 2 years is unknown, but the relatively low initial FIX expression may imply a potential risk of loss of long-term efficacy. However, of the 57 cases (3 cases followed for 5 years and 54 cases followed for 3 years), only 1 case (1.7%) loss long-term expression is inconclusive. Long-term follow-up is needed, and the results should be submitted and updated after the completion of the HOPE-B 5-year study report. (Source: Response #1, HOPE-B CSR, Laboratory measurements, Table 3.3.3)

In response of CDE's clinical IR, the applicant provided CT-AMT-061-02/ad hoc Table 128.21 for the incidence of ALT or AST elevations in subjects with a history of HBV or HCV. Post-dose ALT / AST elevations were not more frequent in subjects with a history of either HBV or HCV compared to subjects that were HCBV(-). At Month 24 pos-tdose, the mean (± SD) uncontaminated FIX activity in HCBV(+) subjects, measured by one-stage aPTT assay, was 40.22 (± 20.83) compared to 31.32 (± 14.63) in HCBV(-) subjects.
Table 24. AST/ALT elevation by HCV/HBV status

	Post-Treatment Period		
	ALT/AST Elevation (N=14)	Other (N=40)	Total (N=54)
Hepatitis Status			
HBV + [a]	1 (11.1)	8 (88.9)	9
HBV -	13 (28.9)	32 (71.1)	45
HCV + [b]	6 (19.4)	25 (80.6)	31
Prior Infection	4 (14.3)	24 (85.7)	28
Ongoing	2 (66.7)	1 (33.3)	3
HCV -	8 (34.8)	15 (65.2)	23

Hypersensitivity and Infusion-Related Reactions

7 subjects had TEAEs of special notification related to IMP administration related to the administration of the investigational product in the ISS population (infusion-related reaction (2 [3.5%]), hypersensitivity (1 [1.8%]), infusion site reaction (1 [1.8%]), etc.). 3 of these subjects required dose interruption. Of these subjects, 5 subjects with positive pre-existing baseline anti-AAV5 NAb.

The applicant submitted a new Table 128.20 Hypersensitivity/Anaphylactic SMQ result, 10 subjects (17.5%) reported hypersensitivity (SMQ) without a subject matching anaphylactic SMQ. Most events were mild in nature. By reviewer's assess, there were 2 subjects (3.5%) with moderate/severe hypersensitivity reaction. One subject [redacted] received only 10% dose of Hemgenix due to severe hypersensitivity reaction requiring epinephrine and vena. One subject [redacted] subject experienced tightness of throat, right neck itching during infusion.

Thrombosis and Thromboembolic events

In CT-AMT-061-02, 3 subjects were identified as potentially having a thromboembolic event. These were PTs of Angina Pectoris (2 events), Peripheral Arterial Occlusive Disease (1 event), and Transient Ischemic Attack (1 event, described in the SAE section above). These subjects had multiple risk factors and were over 65 years of age, so the investigator considered the events unlikely to be related to Hemgenix.

There were 2 subjects who had highest FIX levels >100%. One subject who had a FIX activity level of 122.9% at a single time point post-dose (month 18); his FIX activity level was 45% at month 24. This subject did not have a thrombotic event at 24 months post-dose. One subject [redacted] had FIX activity levels > 100% from month 7 (July 8, 2020) through month 18 post-dose (June 14, 2021) with the highest FIX activity of 121.3%. The subject had a PAOD event on study day 541. He is 70 years old and had several risk factors (i.e. hypertension, aortoiliac atherosclerosis). No subject had supraphysiologic FIX activity (>150%). (Source: Response #4 i)

[Reviewer's Comment]: Although it cannot be completely excluded that this product predisposes patients to the original risk of thromboembolic events, it was agreed that the applicant's conclusion that these events were unlikely to be related to the product and that the overall risk of thromboembolic events was acceptable, as no supraphysiologic FIX levels were observed in the clinical trials. However, in practice, hemophiliacs are considered to be less prone to develop thromboembolic events, but the risk of thromboembolic events after use of this product will be higher than the initial risk (or "return" to the risk of the general population), especially in people with other risk factors, who still need special attention. Therefore, it is still recommended to provide relevant risk monitoring recommendations in the label and RMP. Currently, the applicant has provided relevant information in the draft RMP (e.g. HCP guide) but not in the label, it is recommended to provide this risk information with the label.

Vital Sign

Administration of Hemgenix was associated with a small (≤ 3 mmHg) transient decrease in mean systolic blood pressure within 3 hours post-dose. In ISS population, no clinically significant vital signs abnormalities were reported.

1 AE of Pyrexia (Fever, Study Day 1, infusion-related reaction) were considered treatment-related. 2 subjects experienced tachycardia on Study Day 1, and suspected infusion related.

[Reviewer's Comment]: no significant safety concerns according to the review of the vital signs.

Immunogenicity

No FIX inhibitors were observed after Hemgenix injection.

Safety in Special Population

Subjects with Positive/Negative baseline anti-AAV5 NAb

The 33 subjects who were seronegative for anti-AAV5 NAbs at baseline experienced 325 TEAEs; the most common non-laboratory TEAEs included Headache (36.4% of subjects), Arthralgia (33.3%), Fatigue (27.3%), Nasopharyngitis (24.2%), COVID-19 (21.2%), Toothache (18.2%), Back Pain (15.2%), and Hypertension (15.2%).

The 24 subjects who were seropositive for anti-AAV5 NAbs at baseline experienced 288 TEAEs; the most common non-laboratory TEAEs included Arthralgia (41.7%), Nasopharyngitis (29.2%), Headache (25.0%), Back Pain (25.0%), Pain in Extremity (25.0%), Fatigue (20.8%), Influenza-like Illness (16.7%), Diarrhea (16.7%), Nausea (16.7%), and Oropharyngeal Pain (16.7%).

Of the 33 subjects in the ISS Safety Population who were seronegative for anti-AAV5 NAb, 6 (18.2%) subjects experienced 6 SAEs. Of the 24 subjects who were seropositive for anti-AAV5 NAb, 9 (37.5%) subjects experienced 12 SAEs. Although there was numerical higher proportion SAE in anti-AAV5 positive subjects, all SAE events in baseline anti-AAV5 positive subjects were unlikely related to product.

[Reviewer's Comment]: Overall, there was no clear difference in AEs between subjects with positive anti-AAV5 NAb titers and negative anti-AAV5 NAb titers at baseline, with the exception of one subject with a high baseline NAb titer of 1:3212 and no FIX expression who experienced increased bleeding events. Another exception was infusion-related reactions, as discussed in the AESI section above, 5/7 subjects who experienced infusion reactions were positive for anti-AAV5 NAbs at baseline (1:23-1:3212), although the most events were mild.

Elderly

Of the 57 subjects treated with Hemgenix, 7 subjects were ≥ 65 years of age, safety summary by age group is shown in the Table below.

Table 25. Overview of Safety by Age

	Post-Treatment Period			
	Age >= 65 (N=7; PY=14.5[a])		Age < 65 (N=50; PY=109.5[a])	
	n (%)	E [E per 100PY]	n (%)	E [E per 100PY]
Subjects with at Least one Treatment Emergent AE [b]	7 (100.0)	99 [682.9]	50 (100.0)	514 [469.2]
Subjects With Treatment Emergent Serious AEs	6 (85.7)	9 [62.1]	9 (18.0)	9 [8.2]
Subjects With Treatment Emergent AEs Leading to Death	1 (14.3)	1 [6.9]	0	
Subjects With Treatment Emergent AEs Leading to Premature Treatment Discontinuation	1 (14.3)	1 [6.9]	0	

Source: response #4 iii. Table 128.12

More SAEs were observed in the elderly population, which were all considered not related to study drug, and may be expected in elderly patients.

Most common TEAEs by PT in ≥ 65 years (n≥ 2/7 subjects) included: abdominal pain upper, angina pectoris, Afib, back pain, cystitis, fatigue, hemorrhoid, hypertension, influenza.
[Reviewer's Note]: Considering the pharmacodynamic properties of the product and the nature of the disease, such a number of elderly subjects' data and the same dose recommendation was acceptable, but due to the limited number, it should be stated in the labeling instead of the current wording "no meaningful difference".

Safety Conclusion
Overall, the safety profile of AMT-061 is acceptable. Most subjects experienced mild or moderate AEs. A few subjects experienced infusion-related reactions during infusion and elevated transaminases that may require treatment with steroids. There was one subject who developed HCC and the relationship to AAV was not been established after multiple analyses. However, due to the nature of the product targeting expression in the liver, there was still a potential risk of HCC. Long-term follow-up and labeling warning of the potential risk of HCC were still needed. In addition, there is a high titer positive pre-existing NAb positive subject who experienced no endogenous FIX expression and therefore generate bleeding risk, but the exact NAb threshold were not currently established. In summary, the applicant would request to submit the final results of the CT-AMT-061-02 study and the 15-year long-term extension study CSL222_4001 (interim analysis report and final report) for long-term safety. And request updated labeling once CSL222_3005 study shows result. The RMP will be required to confirm that relevant safety information is clearly understood by healthcare providers and patients.

<Secondary Reviewer's Note>
Based on the applicant response to CDE's IR, there is no plan to enroll subjects from Taiwan into the post-marketing long-term observational study CSL222_4001.

7.5 Ethnic Difference Evaluation
Two subjects of Asian participated in the clinical trials of Hemgenix. Both subjects demonstrated an acceptable efficacy and FIX response. Subject [redacted] who had baseline anti-AAV5 NAb levels of 558.3, demonstrated an ABR of 1.26 during the lead-in period and 0 during the post-treatment period (Month 7-24). The FIX activity demonstrated a notable increase from the baseline of 1% (OSA) to the 18th month, reaching 26%. One subject [redacted] baseline anti-AAV5 NAb 481.9) exhibited an ABR in the lead-in period of 10.67 and an ABR in the post-treatment period (Month 7-24) of 0. The FIX activity demonstrated a increase from the baseline of 2% to 17% by the Month 18.
The applicant submitted new Table 128.10 and Table 128.5, post-hoc subgroup analysis by BMI. ABR demonstrated consistent results across different BMI subgroups. FIX activity exhibited a slight trend of increase with elevated BMI. The lowest BMI subgroup (18.5-24) still demonstrated mean FIX activity of approximately 30%.
Table 26. FIX Activity (OSA) by BMI

	BMI < 18.5 (N=0)	18.5 <= BMI < 24 (N=11)	24 <= BMI < 27 (N=21)	27 <= BMI < 30 (N=13)	30 <= BMI < 35 (N=5)	35 <= BMI < 40 (N=2)	BMI >= 40 (N=1)
Baseline [a]							
n	0	11	21	13	5	2	1
Mean (SD)		1.36 (0.505)	1.19 (0.402)	1.00 (0.000)	1.20 (0.447)	1.50 (0.707)	1.00 (-)
Week 6							
n	0	9	17	13	5	2	1
Mean (SD)		24.51 (9.226)	31.86 (16.470)	37.13 (14.622)	39.98 (7.822)	26.00 (26.729)	24.00 (-)
Month 3							
n	0	10	20	13	5	2	1
Mean (SD)		25.76 (11.870)	35.63 (18.324)	45.67 (20.290)	43.84 (10.330)	27.70 (28.426)	37.50 (-)
Month 6							
n	0	10	21	13	5	1	1
Mean (SD)		29.33 (15.025)	37.53 (20.319)	45.81 (20.977)	44.86 (6.555)	42.60 (-)	42.60 (-)
Month 12							
n	0	9	20	13	5	2	1
Mean (SD)		32.58 (18.733)	40.08 (23.697)	46.81 (23.097)	54.52 (5.277)	28.55 (28.355)	41.10 (-)
Month 18							
n	0	10	21	12	5	1	1
Mean (SD)		28.11 (14.174)	35.16 (20.315)	43.33 (30.927)	46.06 (4.672)	39.80 (-)	35.60 (-)
Month 24							
n	0	10	21	12	5	1	1
Mean (SD)		29.25 (15.937)	35.90 (20.516)	40.00 (22.538)	47.40 (2.871)	33.50 (-)	35.80 (-)

"Uncontaminated" means that the blood sampling did not occur within 5 half-lives of exogenous factor IX use. Both the date and time of the exogenous factor IX use and the blood sampling are considered in determining contamination.

[Reviewer's Comment]: There were only 2 Asian subjects data submitted by the sponsor and it is unable to confirm whether the two Asians are East Asian or not. At this time, clinical data from East Asians is limited. Given that clinical bleeding is primarily dependent on the degree of FIX deficiency, it is expected that race-related differences in efficacy will be minimal for the same FIX level. Based on previous experience with a similar gene therapy product (e.g. AAV-5 based gene therapy, ROCTAVIAN) and the pharmacodynamic nature of Hemgenix, ethnic differences are expected to be negligible. The applicant has indicated that there are currently two ongoing studies in East Asia (CSL222_3002, Japan, initiated September 2023). CSL222_3005 is a multinational study that includes Taiwan (Protocol approved. [redacted] and was initiated in 2024/1. Overall, the bridging study could be Conditional waive with PMR of CSL222_3005 and CSL222_3002 study.

7.6 Post-marketing safety updates
NA

7.7 External Communication (deficiency/inquiry and supplement, expert consultation…etc.)

The applicant's responses to the supplementary information have been included in the relevant chapters of the report.

7.8 Conclusion and Recommendation

Risk benefit assessment

Dimension	Evidence & Uncertainties	Conclusions & Reasons
Analysis of Condition	Hemophilia B is a hereditary disorder characterized by recurrent bleeding due to factor IX(FIX) deficiency. Severe HB is under risk of life-threatening before adulthood. Recurrent joint bleeding can lead to long-term functional impairment. In severe hemophilia B patient, prophylaxis with FIX injections initiated at an early age is the standard of care.	Severe hemophilia B is a life-threatening disease. Hemophilia B has the potential to significantly impair an individual's physical and psychosocial well-being.

Current Treatment Options	At present, the prophylactic treatment for hemophilia B in Taiwan can be administered via intravenous injections/infusion of either a standard half-life FIX product or an extended half-life product. The replacement FIX treatment requires a lifelong regimen of injections, ranging from twice weekly to approximately a two-week interval, depending on the individual's bleeding condition.	Lifelong, repetitive drug injections are a big burden for patients.
Benefit	CT-AMT-061-02(HOPE-B) study showed adequate efficacy of Hemgenix from ABR result, mean ABR during months 7-18 with Hemgenix treatment was 1.51 [95% CI:0.81, 2.82] compared to a mean of 4,19 [95%CI: 3.22, 5.45] during the lead-in period. Mean FIX activity (one-stage aPTT-based) from baseline 1.19(0.39) to 36.9(21.40) at Month 18.	ABR clinical data show a statistically significant and clinically meaningful reduction in bleeding frequency. Endogenous FIX activity also reached clinically relevant levels. Updated efficacy data at 36 months also demonstrated sustained therapeutic benefit.
Risk	The most common adverse reactions with AMT-061 were elevated ALT/AST, headache, blood creatine kinase elevations, flu-like symptoms, infusion-related reactions, malaise, and fatigue. The most significant short-term safety concerns are potential infusion-related reaction and elevated transaminase. 9 subjects with elevated liver enzymes used corticosteroids for a mean of 79.8 days (range: 51 to 130). Other potential risk included HCC, loss of serious risk of bleeding due to lack of pharmacological effect in subjects with pre-existing NAbs.	Hemgenix has an acceptable safety profile and the risks are addressed in the labeling. There are still uncertainties regarding long-term safety and pre-existing NAb threshold. There was 1 PMRs study and a 15-year long-term study. RMP is also required for Hemgenix.

The overall benefit risk assessment is favorable and the clinical review team recommends regular approval of Hemgenix for the indication:

1. “Hemgenix is indicated for the treatment of adults aged 18 and older with severe and moderately severe Hemophilia B requiring Factor IX prophylaxis therapy, without a history of Factor IX inhibitors, and with pre-existing neutralizing antibodies to adeno-associated virus serotype 5(AAV5) titer below 1:900.”
2. The recommended dosage is 2×10^{13} genome copies (gc) per kilogram of body weight, administered as a single intravenous infusion.
3. The stability results support the shelf life of the drug product protected from light at 5 ± 3℃ for 18 months.
4. Pre-approval requirements: The manufacturer must design and develop a comprehensive plan for conducting post-approval AAV5 neutralizing antibody

testing in the domestic market for the purpose of screening patients eligible to receive this therapeutic product.

5. Post-approval requirements:
   (1) Final clinical study report upon completion of CT-AMT-061-02 clinical trial.
   (2) Final Clinical Study Report upon completion of CSL222_3002 in Japan.
   (3) Final Clinical Study Report upon completion of CSL222_3005.
   (4) For the CSL222_3003 trial, the clinical trial report should be submitted according to the timeframe specified in the EMA Risk Management Plan (RMP).
   (5) For the post-marketing long-term observational study CSL222_4001, the clinical study report should be submitted in accordance with the timeframe specified in the EMA Risk Management Plan (RMP).
6. A domestic risk management program shall be implemented, which should include long-term follow-up of domestic patients receiving the treated product.

This gene therapy product was submitted to the Regenerative Medicine Advisory Board for review, as required by regulation, to discuss whether the proposed domestic research plans meet regulatory requirements, and the appropriateness of the indication restrictions regarding existing AAV5 neutralizing antibody titers.

8. CDE final recommendations

This multidisciplinary review recommends approval for [Hemgenix®] (Etranacogene dezaparvovec) for the indication of [Treatment of adults aged 18 and older with severe and moderately severe Hemophilia B requiring Factor IX prophylaxis therapy, without a history of Factor IX inhibitors, and with pre-existing neutralizing antibodies to adeno-associated virus serotype 5(AAV5) titer below 1:900].

Risk Management Plan ■ Required □ Not required
Post-marketing Requirement ■ Yes □ No