EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

23 June 2022
EMA/685615/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Roctavian

International non-proprietary name: valoctocogene roxaparvovec

Procedure No. EMEA/H/C/005830/0000

Note

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

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List of abbreviations

AAV	adeno-associated virus
ABR	annualised bleeding rate
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
AIS	alternative immunosuppressive agents
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
ART	antiretroviral therapy
AST	aspartate aminotransferase
BLOQ	below the lower limit of quantification
BU	Bethesda Unit
CI	confidence interval
CID	clinically important difference
CPK	creatinine phosphokinase
CRP	C-reactive protein
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRB	Data Review Board
ECG	electrocardiogram
eCRF	electronic case report form
ED	exposure days
EOSI	events of special interest
ETV	early termination visit
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	coagulation factor VIII
FXa	coagulation factor Xa
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
HA	Haemophilia A
HAL	Haemophilia Activities List
HAART	highly active antiretroviral therapy
hFVIII	human coagulation factor VIII
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICF	informed consent form
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee
IND	Investigational New Drug (application)
INR	international normalised ratio
IP	investigational product
IRB	institutional review board
ITR	inverted terminal repeat
ITT	intent-to-treat
IV	intravenous
LDH	lactate dehydrogenase
LLOQ	lower limit of quantification
LOCF	last observation carried forward
LT	liver test
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction PD pharmacodynamics
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics

PRO	patient-reported outcome
PROBE	Patient Reported Outcomes, Burdens, and Experiences
PT	preferred term
rAAV	recombinant adeno-associated virus
rhFVIII	recombinant human FVIII protein
SAE	serious adverse event
SAP	statistical analysis plan
SDR	source data review
SDV	source data verification
SFU	spot forming units
SMQ	standardised MedDRA queries
SOC	system organ class
Tab	total antibodies
TEAE	treatment-emergent adverse event
TI	transduction inhibition
ULN	upper limit of normal
vg	vector genomes
VWF:Ag	von Willebrand factor Antigen
WPAI+CIQ+HS	Work Productivity and Activity Impairment plus Classroom Impairment Questions+ Hemophilia Specific

1. Background information on the procedure

1.1. Submission of the dossier

The applicant BioMarin International Limited submitted on 25 June 2021 an application for marketing authorisation to the European Medicines Agency (EMA) for Roctavian, through the centralised procedure falling within the Article 3(1) and point 1a of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 29 January 2021.

Roctavian, was designated as an orphan medicinal product EU/ 3/16/1622 on 21 March 2016 in the following condition: Treatment of haemophilia A.

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

The applicant applied for the following indication: for the treatment of severe haemophilia A (congenital factor VIII deficiency) in adult patients without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5).

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0148/2021 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0148/2021 was not yet completed as some measures were deferred.

The PDCO issued an opinion on compliance for the PIP EMEA-C2-002427-PIP01-18-M01.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's requests for consideration

1.5.1. Conditional marketing authorisation and accelerated assessment

The applicant initially requested consideration of its application for a full marketing authorisation but during the procedure, in response to CAT concerns on the comprehensiveness of the data as a major objection, the applicant requested consideration as a conditional marketing authorisation in accordance with Article 14-a of Regulation (EC) No 726/2004.

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004. However, during the procedure, in view of the major objections, the timetable was switched to standard timelines.

1.5.2. New active substance status

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

1.6. PRIME

Roctavian was granted eligibility to PRIME on 27 January 2017 in the following indication: Treatment of Haemophilia A.

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

- The unmet need is justified in particular on the basis of breakthrough bleeds in prophylactic treatment settings, leading to sequelae including haemophilic arthropathy;
- The potential to address the need is justified on the basis of preliminary clinical data in affected patients, supporting that a single IV administration results in sustained restoration of factor VIII activity, reduction of Annualised Bleeding Rates and improved quality of life.

Upon granting of eligibility to PRIME, Violaine Closson-Carella was appointed by the CAT as rapporteur.

A kick-off meeting was held on 10 May 2017. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

- Strategy for comparability of drug product material from manufacturing processes C, D and D' and qualification of the proposed assay for product strength;
- Adequacy of a conditional marketing authorisation application and need for post-authorisation data generation;
- Paediatric Investigation Plan.

1.7. Protocol assistance

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

Date	Reference	SAWP co-ordinators

18 May 2017	EMEA/H/SA/3546/1/2017/PA/ADT/PR/III	Armando Magrelli, Rune Kjeken
31 May 2018	EMEA/H/SA/3546/1/FU/1/2018/PA/ADT/PR/III	Alexandre Moreau, Rune Kjeken
28 June 2018	EMEA/H/SA/3546/1/FU/2/2018/PA/ADT/I	Alexandre Moreau, Rune Kjeken

The Protocol assistance pertained to the following *quality, non-clinical, and clinical* aspects:

- The overall process and product analytical control strategy; the approach to describe the final commercial manufacturing process in the MAA and provide supportive data during review.
- The proposed comparability plan for the process change.
- Sufficiency of the proposed non-clinical programme to support a MAA.
- The proposed approach to support the clinical development of BMN 270 in paediatric subjects, with respect to timing of study initiation, age cut-off, and the proposed nonclinical studies.
- Adequacy of the proposed safety and efficacy data to support conditional marketing authorisation; the proposed design of the Phase 3 study, including duration, endpoints, and patient population, and whether this study could serve as a confirmatory study; the approach to support the dose selection for the proposed Phase 2 (270-202) and Phase 3 (270-301) studies; the proposed clinical assessment strategy for immunogenicity and vector shedding; adequacy of the proposed overall clinical development programme to enable benefit-risk assessment for a standard MAA; appropriateness of the design of the Phase 3 programme (study 301 and 302) to support a MAA.

1.8. Steps taken for the assessment of the product

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

CAT Rapporteur: Violaine Closson Carella CAT Co-Rapporteur: Ilona G. Reischl

The application was received by the EMA on	25 June 2021
The procedure started on	15 July 2021
The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	4 October 2021
The CAT Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	4 October 2021
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	18 October 2021
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT during the meeting on	28 October 2021
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	5 November 2021
The applicant submitted the responses to the CAT consolidated List of Questions on	15 February 2022
The CAT Rapporteur circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	3 March 2022

The CAT agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	18 March 2022
The applicant submitted the responses to the CAT List of Outstanding Issues on	17 May 2022
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	3 June 2022
An ad-hoc expert group experts was convened to address questions raised by the CHMP on The CAT and CHMP considered the views of the Expert group as presented in the minutes of this meeting.	9 June 2022
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Roctavian on	17 June 2022
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Roctavian on	23 June 2022
Furthermore, the CAT and CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product	17 and 23 June 2022 (respectively)

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The claimed indication of Roctavian (valoctocogene roxaparvovec) is the treatment of adults with severe haemophilia A (congenital factor VIII deficiency) without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5).

Severe haemophilia A is defined by FVIII level <1 IU/dL or <1% of normal.

2.1.2. Epidemiology

HA is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Iorio 2019). It is caused by inherited or de novo mutations in the F8 gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. These mutations can either lead to inadequate production of FVIII or a biologically dysfunctional FVIII, ultimately leading to a defective coagulation process. The clinical phenotype of HA patients can be classified, depending on the level of endogenous FVIII activity, into severe (< 1 IU/dL), moderate (1-5 IU/dL), or mild (5-40 IU/dL).

2.1.3. Aetiology and pathogenesis

Haemophilia A is caused by mutations in the FVIII gene that codes for FVIII protein, an essential cofactor in the coagulation cascade, which diminish or eliminate its effect in promoting normal hemostasis. This gene is located on the X chromosome. Due to this sex-linkage of the disorder, the prominence amongst males is greater than in females especially for severe forms. Although the disorder mainly affects males, females can be carriers of the affected gene and experience mild or moderate symptoms.

2.1.4. Clinical presentation

The natural course of untreated persons with HA (PwHA), particularly those with the severe phenotype, is characterised by lifelong recurrent bleeds into joints, soft tissues, and muscles that lead to painful disabling arthropathy, increased risk of intracranial and other visceral haemorrhage, and early death (Mannucci 2001; Darby 2007). In some patients with moderately severe HA, residual FVIII activity levels between 1-3%, can manifest a severe bleeding phenotype, with 29% of patients requiring FVIII prophylaxis because of a high bleeding frequency (den Uijl 2014).

2.1.5. Management

Factor VIII Prophylaxis

The current standard of care for HA in developed countries has been prophylactic infusion of exogenous FVIII two to three times per week, or episodically (also referred to as on-demand) at the time of a bleeding event (Srivastava 2020). Treatment with existing FVIII replacement products also requires frequent injections, which are associated with complications and a negative impact on HRQoL. The goals of prophylaxis with FVIII are to increase trough FVIII activity to at least a moderate level (1–5 IU/dL) and hence reduce the occurrence of bleeding episodes and subsequent joint damage (Manco-Johnson 2007). Extended half-life (EHL) FVIII products have been engineered to reduce the clearance rate of exogenously administered FVIII (e.g., by developing FVIII-Fc fusion proteins or via conjugation to polyethylene glycol), thereby increasing their half-lives to 18-19 hours, compared to 12-18 hours for conventional FVIII products. This half-life extension permits both a reduction in the number and frequency of injections required to achieve a similar degree of clinical efficacy compared to standard half-life FVIII concentrates (Berntorp, 2016).

Emicizumab

Emicizumab is a humanised, bispecific monoclonal antibody that binds to both activated factor IX and factor X, thereby mimicking the function of activated FVIII. It was recently approved for the treatment of PwHA with inhibitors in February 2018 then without inhibitors in March 2019. While emicizumab represents an incremental improvement in therapeutic burden over replacement FVIII products (Mahlangu 2018; eg, subcutaneous administration), it is still a chronic treatment that requires repeat administration every 1-4 weeks and FVIII concentrate is still required for management of breakthrough bleeding and with surgery that warrants the need for additional FVIII concentrate use (Shima 2016; Miesbach 2019).

2.2. About the product

BMN 270 is an AAV5 vector-based gene therapy that expresses the SQ form of human FVIII under the control of a hybrid human liver-specific promoter (Sandberg 2001). BMN 270 is delivered by a single intravenous dose and is designed to achieve prolonged expression of active human FVIII in the plasma, synthesised from vector-transduced liver tissue.

2.3. Type of Application and aspects on development

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

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

During the procedure, the applicant requested consideration of its application for a conditional marketing authorisation in accordance with Article 14-a of the above-mentioned Regulation, based on the following criteria:

- The benefit-risk balance is positive.
- It is likely that the applicant will be able to provide comprehensive data. The applicant committed to follow patients in study 270-301 for the entire study duration (ie, 5 years) and will provide the requested 5 years of follow-up data from the pivotal study 270-301 to further substantiate the durability of the efficacy. Moreover, BioMarin commits to further understand and refine the corticosteroid (CS) regimen as appropriate based on all emerging data.
- Unmet medical needs will be addressed. Haemophilia A (HA) is a hereditary, serious, and life-threatening disease that can have debilitating health related quality of life (HRQoL) impacts. Despite advancements in the management of HA, there remains an unmet medical need since available treatment options, including non-FVIII replacement therapies (ie, emicizumab), all require long-term, chronic treatments with a high degree of compliance to the prescribed treatment schedule to be effective. Roctavian represents a transformative treatment option for adults with severe HA without FVIII inhibitors who do not have detectable antibodies to AAV5. A single dose of Roctavian will make it possible for patients with severe HA to substantially reduce their bleeding risk, thus relieving them of their treatment burden relative to currently available HA therapies, whilst improving HRQoL over multiple years. This is the first gene therapy to demonstrate superiority against standard-of-care FVIII prophylaxis in reducing ABR. BMN 270 will provide an alternative, effective, and less burdensome treatment option for patients with severe HA.
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. The applicant claimed that making Roctavian available to patients while the collection of comprehensive efficacy and safety data will be ongoing is not expected to represent a risk to public health. Roctavian will provide a transformative, alternative, effective, and less burdensome treatment option for patients with severe HA. As such, the immediate availability on the market of Roctavian outweighs the risk inherent in the fact that additional data are still required.

2.4. Quality aspects

2.4.1. Introduction

Roctavian is a gene therapy medicinal product. The active substance valoctocogene roxaparvovec is a non-replicating recombinant adeno-associated virus serotype AAV5 based vector containing the cDNA of the B-domain deleted SQ form of human coagulation factor VIII gene under the control of a liver-

specific promoter. The expressed hFVIII SQ replaces the missing coagulation factor VIII needed for effective haemostasis.

Roctavian is presented as a solution for infusion containing 2x1013 vector genomes (vg) per mL (strength) of valoctocogene roxaparvovec.

Valoctocogene roxaparvovec is formulated with disodium phosphate dodecahydrate, mannitol, poloxamer 188, sodium chloride, sodium dihydrogen phosphate dihydrate and water for injections.

Roctavian is presented in a 10 mL single-use vial (cyclic olefin polymer plastic resin) with a stopper (chlorobutyl rubber with fluoropolymer coating), crimp seal (aluminium) and flip off cap (polypropylene) containing 8 mL of solution for infusion. Each carton contains 1 vial.

2.4.2. Active Substance

2.4.2.1. General information

AAV5 hFVIII-SQ is a non-replicating recombinant vector. The vector genome is contained within an icosahedral capsid composed of three AAV structural proteins, VP1, VP2, and VP3, of approximately 25 nm in diameter. While VP2 and VP3 are essential for capsid formation, VP1 is essential for infectivity of the capsid. VP1 contains a phospholipase A2 (PLA2) domain required for endosomal escape of the capsid and subsequent trafficking to the nucleus. The amino acid sequence of AAV5 capsid protein is provided.

The vector genome includes double-stranded inverted terminal repeats (ITRs) at its 5’ and 3’ ends and single-stranded DNA encoding a hybrid human liver-specific promoter (HLP), a BDD hFVIII gene and a synthetic polyadenylation signal. A description of the design of the vector is provided.

The hFVIII-SQ coding DNA sequence includes a codon optimised nucleic acid sequence encoding the A1, A2, A3, C1 and C2 FVIII protein domains. The wild-type B domain between the A2 and A3 domains is replaced by a 14 amino acid “SQ” linker sequence, from the normal B domain sequence. The resulting amino acid sequence is identical to that of the human FVIII reference sequence within the heavy and light chains of the protein.

The structure and properties of AAV5 hFVIII-SQ are sufficiently described.

Manufacture, characterisation and process controls

Manufacturing process and process controls

The active substance is manufactured at BioMarin Pharmaceutical Inc. (BPI), Novato Campus, 46 Galli Drive, Novato, CA 94949, USA.

Satisfactory evidence of EU GMP compliance was provided for all sites involved in manufacture, controls and storage of the master cell bank (MCB), working cell bank (WCB) and active substance.

AAV5-hFVIII-SQ is manufactured by co-infection of Spodoptera frugiperda (Sf9) insect cells with recombinant baculovirus (rBV).

The commercial manufacturing process for the formulated bulk active substance (FBDS) consists of cell culture, harvest and purification.

Material is then formulated resulting in FBDS.

Animal-derived materials are not used in the manufacture of AAV5-hFVIII-SQ.

Reprocessing is described in the dossier.

Control of materials

Genetic starting materials

The manufacture of the genetic materials coding for hFVIII, rep and cap is performed in accordance with EU GMP. The manufacturing process is clearly described and raw materials are listed.

Genetic material generation is described in sufficient detail.

Sf9 cell banks

A banking system has been established. The history and the generation of the Sf9 cell line are clearly described and the qualification programme for future production is appropriate. Preparation of Sf9 banks, and of cells at the limit of cell age is presented in sufficient detail and their testing complies with ICH Q5D guideline and Ph. Eur. monograph 5.2.3. Specifications for future cell banks are provided. The stability programme is acceptable and stability data presented are satisfactory.

Raw materials

The qualification of raw materials suppliers and the risk-based qualification is suitable to ensure the quality of raw materials for manufacturing of the active substance.

Control of critical steps and intermediates

Control of critical steps and process material is achieved using process parameter controls and in-process testing. In-process testing is presented, the methods are described, and justification is provided for the defined limits and acceptance criteria. Procedures used for both in-process controls and FBDS release were validated. Control culture testing complies with Ph. Eur. 5.14 and is satisfactory.

Control of critical steps and intermediates is considered appropriate.

Process validation

Four process validation batches have been manufactured according to the commercial process. Results of process parameters and performance attributes are presented for all steps of the process.

Results of cell culture, transfection and harvest steps comply with targets and expected ranges. At the purification stage, most results fall within expected ranges.

Clearance of product- and process-related impurities was studied during process validation lots. The data show acceptable clearance of product-related impurities.

Microbial control during FBDS manufacturing process and hold times is ensured.

Hold times have been validated.

Refiltration of process intermediates and FBDS has been studied. The data met the acceptance criteria.

Overall, the commercial manufacturing process is considered appropriately validated.

Manufacturing process development

Critical process parameters (CPP) and non-CPP are defined for each step of the manufacturing process, based on experiments and risk assessment. The process characterisation studies and data generated to classify the process parameters and define their ranges are summarised in the dossier.

The quality target product profile (QTPP) is presented. Identification of critical quality attributes (CQAs) is based on compendial requirements, threshold of toxicological concern and risk assessment.

Process development history presents the different processes used to manufacture the FBDS and used in clinical studies.

Analytical comparability between FBDS manufactured with previous processes was previously demonstrated and reports are provided.

Analytical comparability of the commercial process to processes used during clinical trials was performed. The data presented indicate that the lots manufactured according to the commercial process are comparable to the lots produced with earlier processes.

Overall, manufacturing process development is considered acceptable.

Characterisation

Genome sequence and amino acid sequence were analysed by sequencing and peptide mapping respectively and demonstrated 100% homology with the reference sequence. Strength, physiochemical properties, and biological activity of the active substance were assessed. The assessment involved several methods used for release or characterisation. The results show that the commercial process leads to a product with the expected strength and physicochemical properties (sequence, secondary structure, particle size, morphology, capsid integrity) and with the ability to generate hFVIII protein with the expected activity.

The potency assay measures transgene activity and transgene expression. It reflects the mechanism of action since it requires binding of the AAV vector to the cells, cell infection and intracellular transfer of the DNA, expression and secretion of hFVIII.

Impurities

Impurities were studied on the commercial lots and on the same primary reference lot used for characterisation.

Product- and process-related impurities were thoroughly evaluated. Process-related impurities include the various components of cell culture process, harvest and purification processes. Most impurities are present at levels below the limit of detection/quantitation (LOD/LOQ) or at very low levels. Characterisation of DNA impurities is extensive.

2.4.2.2. Specification

Specification

The FBDS release and shelf-life specifications include the relevant parameters to assess the quality of the AAV vector: identity, strength, purity, potency (the transgene activity assay measuring biologically active FVIII reflects the mechanism of action), and compendial tests.

Analytical procedures

In general, the analytical methods used for release testing of the active substance are correctly described and validated.

Reference standards

A primary reference standard has been established for release, stability and in-process testing. The preparation and qualification of future primary and working references standards is presented.

Batch analyses

Data are provided for all batches manufactured with historical and commercial processes. The results show acceptable consistency and comply with the acceptance criteria applicable at the time.

Container closure system

The container closure system is sufficiently described. Extractable and leachable studies have been performed.

2.4.2.3. Stability

Stability studies of FBDS involve commercial lots and historical lots.

The tested parameters include relevant quality attributes for potency, strength, purity and general quality attributes that are considered stability indicating. Overall, the proposed shelf life is acceptable.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Description of the product

The finished product is supplied in a 10 mL single-use vial as a clear, colourless to pale yellow sterile solution for intravenous infusion, with a pH of 6.9-7.8 and an osmolarity of 364-445 mOsm/L. The strength is $2x10^{13}$ vg/mL. The contents of multiple vials are combined for each patient dose, depending on the patient's weight.

The primary packaging consists of a cyclic olefin polymer vial with a chlorobutyl rubber stopper, and aluminum crimp seal.

The active substance is formulated with commonly used excipients.

Pharmaceutical development

The overfill volume is adequately justified. Each vial of finished product is filled to allow the withdrawal of 8 mL from each vial.

The justification given for the formulation development is deemed adequate.

The finished product is compatible with the container closure system.

The finished product is also compatible with infusion components. The finished product is stable for 10 hours after completion of thawing including 8 hours of hold time in the syringe and 2 hours of infusion exposed to ambient temperature and light.

The compatibility of FBDS with all contact parts included in BioMarin fill-finish line intended to be used for the commercial finished product manufacturing was confirmed.

As regards determination of hold times, data are presented to support the hold steps.

CPPs were defined at different steps of the manufacturing process.

Overall, the manufacturing process development is adequately described.

Compatibility

When assembling the infusion system, it must be ensured that the components' surface in contact with the Roctavian solution corresponds to compatible materials listed in Table 1.

Table 1 Compatible infusion system component materials

Component	Compatible materials
Syringes for infusion pump	Polypropylene barrel with a synthetic rubber plunger tip
Syringe cap	Polypropylene
Infusion tubing	Polyethylene
In-line filter	Polyvinylidene fluoride filter with a polyvinyl chloride body
Infusion catheter	Polyurethane based polymer
Stopcocks	Polycarbonate
Needles for extraction from vials	Stainless steel

2.4.3.2. Manufacture of the product and process controls

BioMarin International Ltd, Shanbally, Ringaskiddy, County Cork, P43 R298, Ireland is responsible for final EU release. Satisfactory evidence of EU GMP compliance was provided for all sites involved in manufacture, primary and secondary packaging, quality controls and release testing of the finished product.

The batch formula is provided.

The commercial manufacturing process is described.

Control of critical steps and intermediates

IPCs are performed during the finished product manufacturing process.

Overall, the manufacturing process description is adequate.

Process validation

Process validation batches were executed.

For all batches, all CPPs were within the normal operating ranges (NORs).

Finished product manufacturing limits were assessed and were adequately validated. Finished product uniformity was checked.

Process simulation (also known as media fill) runs were performed. All process simulation runs were successful and validate the number of filled vials.

The applicant has given a rationale to justify this maximum batch size.

The justification is deemed acceptable.

Concerning shipping qualification, a summary confirming the physical integrity of the shipping container and vials and the maintenance of the temperature during the transport at ≤ -60^{0}C is provided for finished product and finished goods.

2.4.3.3. Product specification

Specifications

Release and shelf life specifications as well as description of the analytical procedures were provided.

The tested parameters at release contain the elements of identity, strength, potency, purity, safety and general tests of physico-chemical properties.

The proposed release specification is in line with Ph. Eur 5.14 monograph.

Overall, the controls applied to finished product for release and stability are acceptable.

Analytical procedures

The methods only applied for finished product are correctly described.

Characterisation of impurities

One process-related impurity is assessed in the finished product section.

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

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

Reference standard

Reference standards are the same as used to test the FBDS.

Batch analysis

Batch analysis results are presented for historical, clinical and commercial lots.

Container closure system

Reference is made to the text above.

Technical drawings for the different container closure system components have been provided. Adequate specifications are set.

The validation data on vial sterilisation has been provided. This is acceptable.

Information provided on the container closure system is deemed sufficient.

2.4.3.4. Stability of the product

The claimed shelf life for the finished product is 2 years at $\leq$ -60^{0}C.

Stability studies were performed on batches representative of the commercial finished product with respect to manufacturing process and container closure system. Some of these batches were used in clinical studies. The finished product batches were stored at long-term storage and at accelerated storage conditions.

The proposed finished product shelf life of 2 years ($\leq$ -60^{0}C) is acceptable.

A photostability study was performed.

Chemical and physical in-use stability after thawing has been demonstrated for 10 hours at 25°C, including hold time in intact vial, preparation time into the syringes and time for infusion. If necessary, an intact vial (stopper not yet punctured) that has been thawed can be stored refrigerated (2°C to 8°C) for up to 3 days, upright and protected from light. Once thawed the product should not be refrozen.

2.4.3.5. Post-approval change management protocol

A PACMP is submitted to introduce a process change in the finished product manufacturing process. The proposed PACMP is acceptable.

2.4.3.6. Adventitious agents

Analysis of raw materials, cell bank preparation and testing, adventitious agent control by environmental and other controls during manufacturing and viral clearance have been performed for the AAV5-hFVIII-SQ manufacturing process. Potential adventitious agent contamination is controlled through appropriate sourcing and screening of raw materials, testing of the cell banks and genetic starting materials, appropriate equipment cleaning, and a robust system of inactivation, removal, and in-process testing during the manufacturing process. The controls, precautions, testing, and demonstrated clearance of multiple virus types in the manufacturing process collectively demonstrate that the AAV5-hFVIII-SQ manufacturing process is robust and reproducible and provides adequate protection against contamination from adventitious agents in AAV5-hFVIII-SQ FBDS.

No materials of direct human or animal origin are used in the manufacture of AAV5-hFVIII-SQ. Several raw materials of indirect animal origin were used at the early stage.

The information provided for raw materials confirms a negligible risk in relation to adventitious agents.

Cell banks and genetic starting materials used for AAV5-hFVIII-SQ production were screened for adventitious agents contamination, following the principles of Ph. Eur. 5.14 monograph.

The manufacturing process includes efficient steps to clear adventitious viruses according to viral validation studies performed.

Overall, adventitious agents safety is considered sufficiently assured.

2.4.3.7. GMO

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

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

The commercial manufacturing process for the active substance and finished product is adequately described, controlled and validated. Starting materials and raw materials are sufficiently described and their quality is appropriate.

Data from comparability studies between the commercial manufacturing process and the processes used for clinical studies indicate that processes and products are overall comparable. Characterisation of the product is extensive and provides a thorough knowledge of the product.

The specifications include all relevant parameters to assess strength, biological activity, purity and safety of the product.

Protocols for long-term stability studies are satisfactory and no trends are observed.

Adventitious agents safety including TSE has been sufficiently assured.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

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

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

2.4.6. Recommendation(s) for future quality development

None.

2.5. Non-clinical aspects

2.5.1. Introduction

2.5.2. Pharmacology

One in vitro study along with 21 in vivo studies were conducted to evaluate the primary PD of BMN 270 (non-GLP and GLP mouse studies, and non-GLP monkey studies).

All in vivo studies were single dose and used the intravenous (IV) route of administration. With regard to evaluation of the ideal route of administration for mice a comparative study between IV bolus and IV infusion over 30 minutes was conducted in Rag2 -/- mice. Five weeks after administration of nearly equal doses (one high and one low dose) via bolus or infusion hFVIII-SQ protein levels increased in a dose-dependent manner in both routes of administration, however, high SD appeared to be higher in mice administered by infusion as compared to bolus injection. Similarly, FVIII activity was highest in mice administered the high dose by infusion. The levels of hFVIII-SQ DNA (vg/cell) were higher in the infusion group when comparing lower doses, however, were in a comparable range in both high dose groups. RNA levels were again higher in the infusion groups as compared to the bolus groups. In conclusion, administration by infusion appeared to result in higher transfection rates and, as a consequence, higher hFVIII-SQ protein levels and activity. Nevertheless, the applicant chose the bolus route of administration for the ease of use.

All animals used in the nonclinical programme to evaluate BMN 270 were males because > 99% of HA patients are male.

2.5.2.1. Primary pharmacodynamic studies

The primary pharmacodynamics (PD) of Roctavian (BMN 270) was documented in different animal species: two normal mouse strains (C57BL/6 and Crl:CD1(ICR)), an immune deficient Rag2 constitutive knockout mouse model (B6.129S6-Rag2tm1Fwa N12; Rag2-/-) and the haemophilia A knockout mouse crossed with a Rag2-/-mouse model (B6;129S-F8tm1Kaz/J x B6.129S6-Rag2tm1Fwa N12; Rag2-/- x FVIII-/-). Rhesus and Cynomolgus monkeys were also used to characterize PD, biodistribution and toxicity in non-human primates.

The PD endpoints (plasma hFVIII-SQ protein and FVIII activity, liver DNA vector genomes and RNA transcription copies) were evaluated in both mouse and monkey studies. Liver DNA vector genomes and RNA transcription copies were assessed to confirm liver transduction by BMN 270. Plasma hFVIII-SQ protein and activity were used as biomarkers of liver expression of the hFVIII-SQ transgene.

Studies conducted in mice:

In Rag2-/- mice administered $2.0E^{12}$ and $2.0E^{13}$ vg/kg BMN 270, a comparison of bolus and IV infusion was made (Study BMN 270-14-073). For the 2.10^{13} vg/kg dose, an approximate 2-fold increase in hFVIII-SQ protein and FVIII activity was noted with 30-minute infusion versus bolus administration. It appeared that the mode of administration of BMN 270 influences/impacts transgene expression, FVIII

activity and hepatic levels of hFVIII-SQ DNA and RNA. Based on these data, the IV infusion over 30 minutes of BMN 270 was chosen for the further clinical development.

In Study BMN 270-14-072, three doses levels of BMN 270 (2.0E11, 6.0E11, or 2.0E12 vg/kg) were tested in the Rag2-/- x FVIII-/- Mice. These three doses were unable to induce any plasma hFVIII-SQ protein or increase in FVIII activity levels, as both parameters were below the limit of detection. However, hFVIII-SQ DNA was detected in the liver of the treated mice (at 6.0E11 and 2.0E12 vg/kg), proving that vector transduction did occur in liver, but without subsequent protein expression. These first results finally suggested that the three selected doses were suboptimal to induce sufficient plasma hFVIII-SQ protein or FVIII activity levels in this model of mice.

Further to these results, the applicant conducted a second dose ranging study with BMN 270, and tested higher doses: 2.0E12, 6.0E12, 2.0E13, 6.0E13 and 2.0E14 vg/kg in Rag2-/- x FVIII-/- mice (Study BMN 270-14-076). As expected, the first dose giving some detectable expression of hFVIII-SQ protein and increase in FVIII activity (% of normal plasma) was the 2.10^{13} vg/kg dose (actual dose: 1,48.1012 vg/kg), but in this group of animals, only 1 animal out of 10 had detectable levels of hFVIII-SQ protein; and only 2 animals out of 10 had detectable FVIII activity. This underlines that, in the murine model of Rag2-/- x FVIII-/- mice, a threshold of dose is necessary to detect and reach quantifiable and significant hFVIII-SQ expression and increase in FVIII activity.

In Rag2-/- x FVIII-/- mice administered 2.0E13 and 1.0E14 vg/kg BMN 270, a functional assessment of coagulation correction was conducted to verify BMN 270 activity (Study BMN 270-14-074). A dose dependent reduction in blood volume loss and bleeding time was observed at 8-weeks post-dose, even if results displayed important variability. In mice administered 1.0E14 vg/kg BMN 270, blood loss and bleeding time was almost corrected to wild-type levels, comparable to the correction achieved with Xyntha® treatment. This study demonstrated the proof of concept of BMN 270 treatment, showing that it permits to achieve at distance from treatment (in the limit of 8 weeks post-dose) similar efficacy results to those obtained after 30 minutes of infusion of the commercialised recombinant FVIII medicinal product Xyntha®.

In Study BMN 270-14-075, male Rag2-/- x FVIII-/- mice were treated with either 6.10^{12} or 6.10^{13} vg/kg, and were euthanised at week 4 or week 13 post-injection. As already observed in previous studies, the 6E12 vg/kg dose appeared suboptimal and insufficient for obtaining a sustained pharmacological effect. Indeed, even if few levels of hFVIII-SQ protein was detected (in 2/10 mice) at Week 4, no more hFVIII-SQ protein was detected at Week 13. However, male Rag2-/- x FVIII-/- mice that were treated with 6.10^{13} vg/kg exhibited detectable plasma hFVIII-SQ protein at week 4 and week 13. Protein levels increased over time and were 180 ± 100 ng/ml and 366 ± 191ng/ml at 4- and 13-weeks, respectively, indicating that the peak of maximal expression does not occur before the 13 week time point. In liver, hFVIII-SQ DNA levels decreased over time, from week 4 to week 13. Conversely, FVIII-SQ RNA levels increased over time. These overall results suggest that a threshold of 6.10^{13} vg/kg dose is necessary to achieve a sustained pharmacological effect lasting for 13 weeks in male Rag2-/- x FVIII-/- mice. More hFVIII-SQ expression data on a longer period of time would have been appreciated so as to substantiate a sustained phamacological effect, and to highlight a "plateau phase" for hFVIII-SQ expression.

Western blot analysis demonstrated that the expressed plasma hFVIII-SQ was of similar molecular size as rhFVIII-SQ protein (Xyntha®), indicating that despite a truncated genome, expression of both the heavy and light chain of hFVIII-SQ was the correct size most likely due to re-association of complementary strands whereby the truncated termini are then repaired by a DNA-PKcs-independent, Rad51C dependent repair pathway (Hirsch, 2013). These data demonstrate that the circulating plasma light and heavy chains of hFVIIISQ are the same sizes for recombinant FVIII.

Long term expression of hFVIII-SQ was assessed in Rag2-/- x FVIII-/- mice. As Study BMN 270-17-035 was conducted over a 6 month period, it gave first results regarding kinetics of hFVIII-SQ expression over a 24-week period in the Rag2-/-x FVIII-/- mice. At 6E13 vg/kg, significant increases in hFVIII-SQ protein expression and FVIII activity were obtained (compared to 2.10^{13}vg/kg). Levels of hFVIII-SQ protein and FVIII activity were noted to be "fluctuating" between 4 and 24 weeks. At 6E13 vg/kg, the peak of expression of hFVIII-SQ protein seemed to occur around week 12 (maximum of hFVII-SQ expression, and maximum of FVII activity) which corresponds to increasing levels of RNA in liver.

Study BMN 270-14-030 was a GLP pivotal study conducted in normal CD1 mice, and also included biodistribution and toxicity assessment. Study BMN 270-14-030 had the same design than study BMN 270-14-075, but conducted in the Rag2-/- x FVIII-/- mice (same doses, same durations of follow-up – 4 and 13 weeks). During this study, it was noticed that, even at the highest dose of 6.1013 vg/kg, results of hFVIII-SQ expression were quite disparate/irregular at day 90. One animal out of 5 was BLQ for hFVIII-SQ expression, and thus not responding to treatment. This observation raised the notion of "responders" and "non-responders" among individuals treated with BMN 270.

In terms of FVIII activity, data suggested that there was no discernible increase in FVIII activity from murine endogenous levels with BMN 270 administration at 6.10^{12} vg/kg dose level. At 6.10^{13} vg/kg, on Day 90, 3 of 5 mice dosed with BMN 270 had levels of FVIII activity beyond what was expected from endogenous murine FVIII. However, results of FVIII activity were very disparate in this group. It was nonetheless expected to have higher results than controls, as mesured FVIII activity in groups 2 and 3 is supposed to be the sum of endogenous FVIII activity and hFVIII-SQ-related FVIII activity.

An inter-process comparability was conducted in Study BMN 270-16-049; this study had the objective to compare two manufacturing processes: process C (used for phase I/II studies) and process D (used for phase III studies). BMN 270 was administered as a single IV injection at 6.0E12 or 6.0E13 vg/kg to Rag2-/- mice with a 36-day observation period. It appeared that, at the same dose level, the average hFVIII-SQ protein and FVIII activity levels were higher for Phase I/II material compared with Phase III material.

Study BMN 270-16-045 was also a GLP pivotal study. Its aims were to determine the safety and biodistribution of BMN 270 when administered as a single IV bolus dose to wild-type Crl:CD1(ICR) mice with 4-, 13- and 26-week observation periods. In the group 3 (highest dose of 2.10^{14} vg/kg), at day 29, 2 out of 5 mice had vales <MIN QUANT for hFVIII-SQ expression, and values of FVIII-SQ protein expression for the remaining three animals were very disparate (inconsistent), and ranged beteween 3,79 to 218 ng/ml. This finding is also observed at day 92, where 3 mice out of 5 have values <MIN QUANT, despite the fact that this timepoint is suggested to be the maximum of protein expression. The applicant was requested to provide a detailed justification on the heterogeneity in hFVIII-SQ expression in the normal CD1 mice (ie absence of expression or hFVIII-SQ widespread results). Clinical relevance of this finding was also questioned. The applicant specified that the study was conducted in CD1 mice, a immunocompetent mice, thus able to mount an antibody response against a heterologous human protein. Results indicated that animals with higher anti-hFVIII TAb titers had FVIII activity that was BLQ.

In terms of FVIII activity, in group 3 (2.10^{14} vg/kg), for 2 animals out of 5, there was a significant increase in mean FVIII activity on day 91, which correlated with increase in FVIII-SQ expression. On day 182, all five tested animals were <MIN QUANT for FVIII activity. Moreover, two animals in particular (animals 3265 and 3275) displayed significant levels of hFVIII-SQ protein at day 182 (76,2 and 77,4 ng/ml respectively), associated with <MIN QUANT values for FVIII activity.

Two studies were then conducted in the neonatal mice, supporting a future extension of indication in the paediatric population. Study BMN 270-15-088 confirmed that neonatal mice livers were

transducible by rAAV5 vectors as shown by detectable vector genomes and RNA transcripts, with maximum levels of DNA and RNA measured in liver 24 hours post dose. By 1 week, plasma FVIII protein and activity peaked in neonatally-dosed mice and subsided over time. For adult animals, mice had liver DNA levels quite similar to those observed in neonates at 24 hours and 1 week post-dose; but liver RNA levels appeared lower in adult mice than in neonates at these same time points. Conversely to neonates, these levels in adult mice corresponded to very low-to-non-measurable plasma FVIII protein or activity. The applicant was requested to discuss the discrepancies observed in hFVIII-SQ protein expression profile amongst adult and neonate animals, considering that quite the same levels of hFVIII-SQ DNA and RNA are detected in liver of adult mice and neonates at 24h and 1 week post-infusion : The applicant first explained that this observation could be linked to faster capsid trafficking kinetics in the neonatal liver leading to a faster FVIII-SQ transcription in the neonatal liver (data on file), and secondly that adult mice have more than 10 times higher blood volume than neonatal mice, explaining the weaker expression. The applicant also justified the differences in hFVIII expression observed between the 24h time point (undetectable expression) and the 1 week time point, as plasma FVIII-SQ protein concentrations were much higher at 1-week post dosing. This delay of 1 week is explained by the time-lag between the transcribed RNA being translated, correctly folded and secreted into the blood. It is known that FVIII-SQ protein is not easily folded and secreted.

In the second study in neonatal mice (Study BMN 270-17-059), results indicated the importance of the time of treatment (childhood versus adultness) and gave evidence that BMN 270 treatment does not behave the same way in terms of hepatocytes transduction efficacy, RNA expression, FVIII-SQ expression profile regarding the age at the time of treatment. However, as the claimed population is adult patients, both studies are of secondary importance.

A new study conducted in juvenile animals was completed (Study BMN 270-18-012). The objective of this GLP study was to determine the safety of BMN 270 following a single intravenous injection to RAG2-/- x FVIII-/- double knockout (DKO) male mice at different ages and to determine expression profile of hFVIII-SQ as a function of age and post-natal development. The applicant specified that the selected doses were designed to investigate the hFVlll-SQ expression profile at different ages of mice (regardless of their age and weight) and to ensure that all age cohorts received the same number of vector genomes. However, the rational for having designed such similar dosages amongst all groups of age is not well explained/understood. Altogether, these data demonstrated that i) decreases in vector DNA are greater in mice dosed at younger ages, ii) RNA concentrations increased to a greater extent in animals dosed at older ages, iii) by Day 84 post-dose, animals dosed at younger ages appeared to have lower plasma protein concentrations. These results suggest that protein synthesis and secretion may be offset by increasing blood volume in younger mice. Importantly, it was noticed that the applicant did not provide any general synthesis nor discuss the results obtained in terms of hFVIII-SQ expression and DNA and RNA persistence over time, according to liver growth as a function of age and post-natal development.

A discussion on the expression and persistence of hFVIII-SQ DNA/RNA and hFVIII-SQ protein, in a juvenile haemophilic liver, was expected, in light of the risk of loss of transgene expression due to dilution of viral genomes due to rapid cell proliferation. In its response, Biomarin outlined that the question of the optimal age for child treatment with BMN 270 appears complex, as the maturational rate of mice does not linearly correlate with humans. Indeed, it appears that mice dosed at younger ages (ie 1 or 2 weeks) have lower plasma hFVIII-SQ protein, hFVIII-SQ DNA and FVIII activity compared with those dosed at older ages, ie at 6 or 8 weeks. According to Biomarin, the optimal age to administer AAV5-FVIIISQ in mice would be 4 weeks or older as these mice had higher plasma hFVIII-SQ concentration and FVIII activity when dosed at this time. However, data gained in NHP studies showed different relationships between dose-timing and transduction patterns than those observed in mice. Overall, Biomarin announced that it will work and engage with the PDCO at the time

of the submission of an extension of indication in children. For the time being, the application is only sought in adults.

Studies conducted in monkeys:

In cynomolgus monkey administered hFVIII-SQ vectors of two different capsids (AAV5.2 hFVIII-SQ and AAV8.2 hFVIII-SQ), the differences in plasma hFVIII-SQ protein expression was evaluated over 8 weeks after a single IV bolus (Study BMN 270-13-031). Administration of a single injection of AAV5.2 hFVIII-SQ induced detectable levels of human FVIII-SQ by Week 2 post treatment. For both animals treated at 2.10^{13} vg/kg, hFVIII-SQ proteins levels peaked either at week 4 (1 animal) or at week 5 (1 animal), however, hFVIII-SQ protein levels declined hereafter. Of note, in both animals treated with 2.10^{13} vg/kg, Cmax were 22 and 28 ng/ml, at week 5 and week 4, respectively. These concentrations did not lead to significant increases in FVIII activity. All AAV5.2 FVIII-SQ treated monkeys (4 animals) developed anti-AAV5 titers by 1 week post dosing, independent on the dose. The AAV5 capsid was selected for continued development.

In cynomolgus monkeys administered 2E13 or 6E13 vg/kg of two manufacturing lots produced in two cell lines (Baculovirus infected Sf9 insect and human 293T cells); relative plasma hFVIII-SQ protein levels were assessed (Study BMN 270-14-014). Plasma hFVIII-SQ levels increased to reach a Cmax by 4/5 weeks post-dose (for baculo material) or 3 weeks (for 293 material), but they declined thereafter, to reach insignificant levels by weeks 5 to 8. Of note, no sustained expression profile ("plateau phase") was demonstrated in animals given 6.10^{13} vg/kg. For animals 1001 and 1002, dosed with 2.10^{13} vg/kg, only modest to no hFVIII-SQ secretion was obtained. The applicant's opinion is that monkey 1002 did not have efficient liver transduction compared to the rest of the treated monkeys. The applicant also justified that the low protein expression level in animal 1002 was correlated to corresponding low levels of liver hFVIII-SQ DNA and RNA. The low protein expression level in animal 1002 was estimated to be related to a less successful liver transduction compared to animal 1001. The applicant specified that there is an inter-subject variability in AAV-based gene therapy in preclinical and clinical settings, suggesting that animal 1002 did not respond to BMN 270 treatment. This animal did not display an efficient liver transduction, leading to an absence of hFVIII-SQ protein expression. This heterogeneity in liver transduction highlights the limits of this AAV gene therapy.

In cynomolgus and rhesus monkeys administered 6E12 and 2E13 vg/kg BMN 270, relative expression of hFVIII-SQ was assessed over 6 weeks (Study BMN 270-14-062). Plasma hFVIII-SQ levels increased over 4 to 5 weeks but declined thereafter. Cynomolgus monkeys appeared better responders to BMN 270 treatment than Rhesus monkeys. This study highlighted a significant inter-individuals variability, in hFVIII-SQ plamatic expression. No sustained plasmatic protein expression was demonstrated beyond a 4 to 5 weeks period. One Rhesus monkey (animal 3002) did not display any hFVIII-SQ portein expression throughout the study period (6 weeks). These results of low hFVII-SQ protein expression again highlight the high inter-subject variability among treated NHP, and questions the overall liver transduction efficiency in this species. Translation to clinics is questioned.

A non-GLP study of cynomolgus monkeys administered 6E13 vg/kg BMN 270 was conducted to determine the comparative PD of BMN 270 in monkeys with varying baseline AAV5 TAb and TI titre status (Study BMN 270-16-021). Even though animals in all groups received the same dose level, the mean hFVIII-SQ concentration in the group comprised of anti-AAV5 TI+ TAb+ animals was lower than the control group, which was comprised of anti-AAV5 TI- TAb- animals. The applicant was requested to comment on the better results for hFVIII-SQ expression levels obtained in group 2 of animals with transduction inhibitors (TAb-/TI+) versus those obtained in group 1, without transduction inhibitors (TAb-/TI-). The applicant specified that, although animals in Group 2 with detectable inhibitors of AAV5 transduction had higher mean levels of maximal hFVIII expression than control animals (Group 1), this higher mean was attributable to a single animal in Group 2 with higher FVIII expression compared to

other animals. The other 3 animals in Group 2 had similar hFVIII expression levels as the animals in Group 1. In addition, the range of hFVIII-SQ expression in Group 1 animals was within the range of expression in Group 2 animals (without considering animal 3002). The applicant underlined that the study confirms that subjects with low AAV5 TI titers, and without detectable AAV5 antibodies (as in Groups 2 and 3), may optimally respond to AAV5 mediated gene therapy.

These findings suggested an association between pre-existing neutralizing anti-AAV5 TAbs and reduced human FVIII-SQ levels. No evidence for such association was found between pre-existing anti-AAV5 TI in the absence of anti-AAV5 TAbs. However, the data suggested that some subjects with pre-existing neutralizing anti-AAV5 TAbs can remain responsive, since three of five animals in group 4 had detectable FVIII-SQ in plasma, albeit at reduced levels in two of these three animals.

In Study BMN 270-16-046, male cynomolgus monkeys screened negative for anti-AAV5 TAb and TI were given BMN 270 v2.0 and BMN 270 v3, by a single IV bolus injection. The secondary objective examined the chronic PD response when monkeys dosed with each construct were also treated with a B-cell depleting antibody (Rituximab) and prednisolone. This study revealed that all dosed animals seroconverted to anti-AAV5 TAb positive by the first assessed time point post dosing, Day 29, including those that received the immune suppression regimen. Subsets of animals dosed with each of the three different FVIII encoding constructs developed anti-hFVIII Tab responses. These responses were not diminished by immune suppression with Rituximab and Methylprednisolone and were not effective at extending detection of FVIII-SQ protein in plasma. Two of 12 cynomolgus monkeys treated with the immune suppression regimen showed sustained FVIII-SQ plasma protein.

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were performed, as pharmacological activity of hFVIII-SQ is restricted to the human coagulation cascade. It is thus not expected that hFVIII-SQ displays other pharmacological effects.

2.5.2.3. Safety pharmacology programme

Safety pharmacology studies were not performed because BMN 270 components (capsid, vector DNA, corresponding RNA and hFVIII-SQ protein) are not expected to impact major organ systems' (central nervous system, cardiovascular, renal or respiratory) function. Toxicity assessments did not reveal particular findings on central nervous system, respiratory, cardiovascular, and renal functions.

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies have been performed, based on the intended use of BMN 270 and the known pharmacology of the transgene product.

2.5.3. Pharmacokinetics

Pharmacokinetics after BMN 270 administration have been studied in three different mouse models (Rag2-/- x FVIII-/- double knockout mice (immunosuppressed and haemophilic phenotype), Rag2-/- mice (only immunosuppressed phenotype) and wild type Crl:CD1(ICR) mice) and in wild-type cynomolgus and rhesus monkeys.

The specific biodistribution of BMN 270 in the liver (ie expression and persistence) was a parameter included and evaluated in the course of 18 PD completed studies in mouse or monkey. Results are mainly presented in the pharmacology section, as they more reflect transgene expression and duration of expression rather than biodistribution of the vector itself. However, some liver biodistribution results

are nonetheless reported in this PK section, so as to inform on the kinetic profile of hFVIII-SQ expression and DNA and RNA clearance over time.

The biodistribution of BMN 270 in the liver, lung, kidney, and brain was evaluated in one completed non-GLP mouse study (Study BMN 270-14-075). The biodistribution of BMN 270 to blood, bone marrow and multiple tissues (brain, kidney, lung, mesenteric lymph node, testis, heart, spleen, and liver) was evaluated in two completed GLP mouse studies (Studies BMN 270-14-030 and BMN 270-16-045).

Per CAT/CHMP request, the biodistribution and elimination of BMN 270 from monkey brain has been specifically evaluated in one completed non-GLP study (Study BMN 270-20-023), which consisted in a comparison of brain biodistribution results taken from two independent conducted studies (studies BMN 270-14-062 and BMN 270-16-046).

The biodistribution of BMN 270 vector outside liver, in non-rodent species, has not been assessed in monkeys, and this appears detrimental.

Methods of analysis: to quantify vector distribution in the whole body, vector genomic DNA or transcript RNA biodistribution in liver and other tissues were measured using qPCR with primers and probes specific for unique sequences in the codon-optimised human FVIII-SQ transgene. Analytical methods were developed for the characterisation of vector DNA and RNA transcription copies in liver and other tissues and organs.

In Study BMN 270-14-075, following a single IV administration via the tail vein of either vehicle, 6.0E12 or 6.0E13 vg/kg BMN 270 to male Rag2-/- x FVIII-/- mice, vector DNA was found in liver, at both 4 weeks and 13 weeks post dose. Liver hFVIII-SQ DNA decreased with time, while liver hFVIII-SQ RNA levels increased with time from 4 to 13 weeks post-dose. Vector DNA (hFVIII-SQ DNA) was also assessed in other organ/tissues such as brain, kidney and lung.

In these tissues/organs, BMN 270 DNA was detected at significant levels within all tissue types for all animals at both day 28 (week 4) and day 91 (week 13), but with decreasing amounts of BMN 270 DNA detected at day 91 compared to day 28 in each tissue, demonstrating a clearance of vector DNA in organs and tissues over time, allowing to estimate that an elimination kinetics is in place in each of these tissues after dosing.

At day 28 (week 4), BMN 270 DNA was detected at the highest level in the lung ($\approx 10^5$ vg/mg tissue) with decreasing amounts of BMN 270 detected in the kidney ($\approx 10^4$ vg/mg tissue) and brain ($\approx 10^3$ vg/mg tissue) (Lungs>Kidneys>Brain). This study confirmed the potential for BMN 270 to migrate and distribute to (other than liver) distant and systemic organs after an IV single injection of 6.0E13 vg/kg (clinical dose).

The objective of Study BMN 270-17-035 was to evaluate hFVIII-SQ protein expression over a 24-week period following a single intravenous administration of BMN 270 in a Rag2-/- x FVIII-/- double knockout mice (doses: 2E13 or 6E13 vg/kg). This study also permitted to evaluate clearance kinetics of DNA and RNA over a 24 weeks period. Liver hFVIII-SQ DNA levels decreased from 4 to 12 to 24 weeks at both dose levels, suggesting a loss of DNA from hepatocytes over time, while liver RNA levels increased and stabilised in the same time.

As '24 weeks' was the last time point assessed, it is unknown if further loss of vector DNA is expected over time. This loss of vector DNA from hepatocytes over time was further detailed. At the first round of the precedent submission (Day 80), the applicant presented data related to the different forms (species) of DNA found in transduced livers in mice. The applicant acknowledged that a true decrease in DNA levels in liver is plausible over a 24 weeks period of time that could be attributable to a loss of truncated linear DNA. Indeed, levels of full length genomes appeared stable from 4 to 24 weeks.

Importantly, a further characterisation of the DNA forms showed that levels of circular full-length and circular ITR-fused genomes remained stable from 4 to 24 weeks. So, according to the applicant, linear DNA may be lost over time while the levels of full-length circular forms remain resistant to host-mediated degradation, thus responsible of long-term efficacy.

An objective of Study BMN 270-14-030 was to evaluate the biodistribution of BMN 270 to blood, bone marrow, liver and other tissues (i.e., brain, kidney, lung, mesenteric lymph node, testes, heart, and spleen) in CD1 mice, after one single IV dose of 6.10^{12} or 6.10^{13} vg/kg. Vector DNA quantities were reported as mean vector genomes/mg of tissue or ml of bone marrow and blood, and as a mean vector genomes by µg of DNA tested.

At day 28 post-infusion, after a single IV injection of 6.1013 vg/kg (selected clinical dose), vector DNA was detected in all tissues assessed, bone marrow, and blood from all animals. Vector DNA was detected in the highest amounts in spleen and liver with decreasing amounts in bone marrow, lymph node, lung, kidney, blood, heart, testis and brain. At day 28, the spleen appeared as the predominantly organ to be perfused by the BMN 270 vector (272% relative to the liver). At day 91, vector DNA was still present in all tissues assessed but DNA levels decreased in almost all of them, except brain, which displayed higher levels that at day 28: This finding of increasing amount of BMN 270 DNA at day 91 was unexpected, and thus challenged:

At the first round of submission of the precedent submission (Day 80), the applicant acknowledged the potential concerns raised by the increasing vector DNA concentrations in brain observed in BMN 270-14-030 between 4 weeks (day 28) and 12 weeks (day 91) post administration in the 6E13 vg/kg dose group. As such, upon making the observation, follow-up investigations were conducted to further characterize the biodistribution of vector DNA in brain. Ultimately, the results of the follow-up investigations led to the conclusion that the concentrations reported at Day 91 with 6E13 vg/kg in BMN 270-14-030 were an aberrant finding. Follow-up investigations included determination of the vector DNA concentration-time course in brain tissue from two additional studies including a completed non-GLP study, BMN 270-14-075, and a subsequent GLP toxicology and biodistribution study, BMN 270-16-045. The observation of increasing vector DNA concentrations with 6E13 vg/kg in BMN 270-14-030 were not observed in any of the four other time courses evaluated. Based on the irreproducibility of the result obtained on Day 91 with the 6E13 vg/kg dose in BMN 270-14-030, the applicant considered it as an aberrant result as concentrations of vector DNA are not believed to increase in brain between 4 and 12 weeks post BMN 270 administration.

Per CAT/CHMP request, results of a new brain biodistribution study are submitted in the course of this new submission (Study BMN 270-20-023): The specific brain biodistribution of BMN 270 DNA was evaluated in one completed non-GLP study in monkey brain (BMN 270-20-023) in which brain retained samples were taken from two previously conducted studies in NHP (BMN 270-14-062 and BMN 270-16-046). Results confirm that BMN 270 DNA is distributed to brain and is present at both sacrifice time-points Day 43 (week 6) and week 13 post injection. It is detrimental that no kinetics data is provided, for a same dose, inside a single study, allowing to objectify elimination kinectics over time from the brain.

Even if data assessed across two independent and separate NHP studies indicate a partial and gradual elimination of DNA genomes from brain tissue over time allowing to think that an elimination kinetics is in place in brain tissue after one unique BMN 270 injection, comparing brain biodistribution data across two different NHP studies is not an ideal situation. The applicant explained that a sole and unique study in NHP, assessing the clearance over time of BMN 270 DNA and RNA was not performed. In consequence, to answer the question raised by Rapporteurs, the applicant used brain tissue data from two different studies, BMN 270-14-062 and BMN 270-16-046, to characterize the distribution and elimination kinetics of BMN 270 in the brain of NHP. Given the reduction in mean average copies/µg

DNA between Day 43 and Week 13, further RNA and protein characterisation was not considered warranted by the applicant.

To note, the applicant did not give an estimation of the delay necessary for a total clearance from brain, although requested; this could have been provided. As no BMN 270-related major safety concern has been reported in CNS at the clinical safety level, this issue is not pursued.

The expression profile of BMN 270 was also determined by quantifying RNA transcripts using a RT-qPCR method. In group 3 (6.1013 vg/kg: selected clinical dose), at day 28, RNA expression was detected in all tissues/organs assessed, but it is acknowledged that protein expression was predominantly seen in liver, with very few amounts detected in other organs/tissues (<1% relative to the liver). RNA transcripts levels increased in liver from day 28 to day 91, highlighting that the maximum of RNA transcripts level does not occur before Day 91. At day 91, at the 6.1013 vg/Kg dose, the hFVIII-SQ RNA level in mice brain was seen to increase compared to the level observed at day 28. Increasing RNA levels in brain over time could have been commented by the applicant (in terms of safety).

Even with a liver-specific promoter included in the vector construction, transgene expression was observed in a large panel of non-targeted organs and tissues, at low levels. This has also been demonstrated by other AAV-mediated gene therapies with transgene expression regulated by tissue-specific promotors (Prasad 2011). Such an illegitimate expression is known, even for chromosomal genes. While the exact level detected, as compared to other cellular genes, is not well defined, such an expression is not expected to be associated to a negative side effect.

In Study BMN 270-16-045, two doses were assessed in the Crl:CD1 mice: 6.1013 and 2.1014 vg/kg. Biodistribution was assessed at day 28, day 91 and day 182 in blood, bone marrow, liver and other tissues (i.e., brain, kidney, lung, mesenteric lymph node, testes, heart, and spleen). At day 28, all tissues/organs showed positive detection for vector DNA. At Day 28, BMN 270 DNA was detected predominantly in the spleen [Spleen>Liver>Bone Marrow>Lung>Mesenteric Lymph Node]. When corrected to vector genomes per mg of tissue, the levels of vector genomes detected in the spleen were about 562% and 111% of those observed in liver (target organ) for dose groups 2 and 3, respectively.

On Day 91 and 182, BMN 270 DNA was detected predominantly in the liver. At day 182, vector DNA was still detected in all tissues, except blood, but clearance of DNA vector was observed in all organs. The highest levels detected in liver, lung and bone marrow. From Day 28 to Day 182, all organs and tissues tested displayed clearance of the vector over time. Of note, at day 182, vector DNA still detected in brain and testis.

CD1 mice testes were still positive for hFVIII-SQ DNA detection at the last study time point (12491 vg/mg of tissue at day 182): as already stated above, this finding questions the delay necessary to obtain a full clearance/elimination of viral DNA from the testes, and the risk for horizontal and vertical germline transmission. To note, sperm and semen have not been tested in both biodistribution studies BMN 270-14-030 and BMN 270-16-045.

As concerns the issue on brain distribution, at both dose levels, brain distribution was observed. But DNA levels were shown to decrease over time for both dosages. BMN 270 DNA was still detected in brain at the last study time point, ie Day 182 post-injection. The delay necessary for a total clearance from brain was not indicated by the applicant. On the contrary, RNA levels were shown to increase over time inside mice brain, until the last study time point.

The applicant emphasised that such an expression is not expected to be associated to a negative side effect. As explained by the applicant, endogenous FVIII is produced primarily in the liver, likely by liver sinusoidal endothelial cells (LSECs; Shahani 2013). However, extra hepatic production occurs and

suffices for hemostasis (Jacquemin 2006). While the extrahepatic sites (of hFVIII production) are not well understood, evidence in literature suggests they include several organs including kidney, spleen, lung and brain (Wion 1985; Hollestelle 2001; Jacquemin 2006), with quantitative analysis of FVIII mRNA levels reporting levels in brain approximately 14% relative to liver in mice (Hollestelle 2001). The applicant emphasised that compared to the relative RNA transcript copies observed in non-target tissue relative to liver in BMN 270-14-030 and BMN 270-16-045, it is possible the low levels of potential BMN 270-derived FVIII protein are less than protein endogenously produced in these tissues in people without haemophilia.

Finally, both studies BMN 270-14-030 and BMN 270-16-045, demonstrated that BMN 270 distribution was predominantly observed first in spleen and liver (target organ), with persistence of vector genomes in all tissues assessed till the last time-point assessed (Day 182). Little to no transcription in non-liver tissues was observed (due to the liver specific HPL promoter).

Several PD studies have been performed in monkeys (Cynomolgus or Rhesus). In monkeys, biodistribution assessment was limited to quantification of hFVIII-SQ DNA and RNA in liver only; these studies did not include quantification of vector genome nor RNA transcripts to distant tissues/organs.

The Study BMN 270-14-014 assessed the relative activity of BMN 270 from two manufacturing lots produced in two cell lines (Baculovirus infected Sf9 insect and human 293 cells) over 8 weeks when given as a single IV administration to cynomolgus monkey. This study was interesting in the fact that it gave evidence of the important variability/disparity in liver transduction between individuals, treated with a same dose.

In Study BMN 270-16-021, the phamacodynamics of BMN 270 was assessed in monkeys depending on transduction inhibition (TI) titre and total antibody (TAb) status in animals. This study indicated that presence of pre-existing antibodies prevent/decreases liver transduction efficiency, as only two animals out of five displayed significant vector genomes/cell inside liver. This study also pointed out the variability/heterogeneity in liver transduction efficiency, as several animals, even if dosed with a same dose, display huge discrepancies in liver transduction efficiency.

Finally, one study assessing the germline transmission potential of BMN 270 was conducted in the male Rag2-/- mice (Study BMN 270-19-008). Rag2-/- male mice were treated with 6.1013 vg/kg BMN 270, and mated with F0 untreated female Rag2-/- mice. F0 male mice were then euthanised at the end of each cohabitation period (ie Day 15 or Day 50 post-injection). F1 offspring were euthanised at 21 days of age. The F1 offspring was assessed for the presence of the hFVIII-SQ DNA in the liver (+/- testes and ovaries). In F1 generation pups, qPCR analysis was done on liver tissue only (and not more organs). The rational for having screened liver only for hFVIIISQ DNA in F1 pups was raised by Rapporteurs:

At the first round of the precedent submission (Day 80), the applicant answered that the liver of the F1 offspring was selected to perform qPCR analysis of F1 offspring because hepatocytes have a relatively long lifespan compared to other cell types, and because data from previous mouse studies dosed with BMN 270, which contains a liver specific promoter, demonstrated long-term retention of transgene DNA in the liver. Successful germline transmission would require the integration of transgene DNA into the sperm genome, resulting in vector expression in every cell of the F1 offspring. There are no relevant examples in the literature of mosaic distribution of transgene DNA (e.g. in lungs but not in liver) as a result of extra-chromosomal vertical transmission followed by cell division during embryonic and fetal development.

The Rag2-/- male F0 mice that received BMN 270 expressed the hFVIII-SQ protein in plasma, and had BMN 270 DNA in their liver and testes both at Study Day 15 and Study Day 50. Levels of hFVIII-SQ DNA in testes were reduced by approximately 2 logs compared to liver samples. However, no

transgene DNA (hFVIII-SQ) was detected in the liver any of the pups (F1) born from by F0 male mice treated with BMN 270. The conclusion of this study was the absence of evidence of germline transmission when assessing the liver of the F1 generation for hFVIII-SQ DNA by qPCR: no transgene DNA was detected in the liver in any of the F1 pups (n = 86) sired by male mice treated with BMN 270.

On the basis that testes were detected positive in qPCR at day 15 and Day 50, the applicant did not specify if they conducted cell fractionation studies on sperm/semen, to investigate whether sperm cells (mature spermatozoa or spermatogonia) were transduced by BMN 270. The question was raised on i) the capacity of BMN 270 to transduce germ cells and ii) the localisation of the hFVIII-SQ DNA, RNA and protein inside testes/sperm/semen.

During the procedure, the applicant answered that cell fractionation studies on sperm were not conducted at the non-clinical level. A question was also raised on the estimation of the delay needed to obtain complete clearance of BMN 270 DNA and BMN 270 transcripts from the testes and sperm/semen from F0 animals. The applicant explained that rat testes from Study BMN 270-16-045 were used to estimate the time to obtain complete clearance following 6E13 vg/kg BMN 270 administration. DNA from testes is estimated to be <LOQ of the assay (<50 vg per µg DNA) at approximately 67 weeks post-dose (ie 1 year and 4 months) (nb: DNA clearance is consistent with a first order elimination according to the applicant).

Another GLP study assessing germline transmission was conducted (Study BMN 270-19-033), so as to increase the number of mice and study power. All F0 generation males administered BMN 270 (Group 2) had detectable BMN 270 DNA in livers and testes, which confirms that all were successfully dosed, and supports the assessment of germline transmission. The applicant transmitted mean hFVIII-SQ DNA values obtained in testes from F0 male mice, harvested on Day 50. However, the applicant should have estimated and given the DNA levels present in the sperm of F0 male animals at the time of mating, and discuss them in light of concentrations observed in the sperm of male patients after a 6 month concentration period which is foreseen in clinics. Again, it was noticed that sperm and semen from F0 parental animals were not tested for hFVIII-SQ DNA detection in this newly submitted study. This absence of analysis (cell fractioning) on the sperm/semen of F0 animals appears detrimental, as more information was needed to know which species/forms of the BMN 270 vector (infectious/noninfectious particles, heavy particles, intermediate particles or free DNA) localize in which compartment of the semen/sperm.

During the procedure, the applicant emphasised that although hFVIII-SQ DNA levels were detected and measured in the testes (and liver) on Day 50, corresponding DNA levels were not measured in the sperm of F0 male animals at the time of mating (Day 38-Day 50). No relationship can thus be drawn between the observed absence of germline transmission in F1 pups at birth, and the level of detection of vector genomes in F0 males' sperm, between Day 38 and day 50 post dosing (time of cohabitation). This information would have nevertheless been of added value. A question was also raised on the nature of the forms/species of the BMN 270 vector present in the semen/sperm after one unique IV injection (infectious/non-infectious particles, full capsids, intermediate capsids, empty capsids, free DNA, etc….) and the risks (horizontal and vertical transmission) associated with.

The applicant explained that i) cell fractionation analysis was not feasible in the course of NHP studies and ii) that this search was only performed in semen from two male patients in the course of clinical study BMN 270-201 (who did not clear transgene DNA from that matrix after 52 weeks), revealing absence of transgene DNA in the sperm. Inside clinical study 270-301, results revealed that potentially infectious particles (ie encapsidated vector DNA) were detectable in semen from 131 of the 133 subjects (98.5%) subjects. These 131 male patients did manage, over time, to clear their semen along with 3 consecutive negative samples. The time/delay necessary to obtain the first of the three consecutive negative semen samples was estimated: the median time [min, max] to obtain the first of

3 consecutive negatives was 3.00 weeks [0.429, 12.1]. It is hence understood that the 131 patients had their first sperm negative sample with a maximum delay of 12 weeks.

In this new submitted study (with the same study design), all F1 pups (53 male and female animals) were negative for the presence of hFVIII-SQ DNA in liver tissue, indicating that germline transmission did not occur between the F0 and F1 generations.

In summary, hFVIII-SQ DNA was not detected in any of the F1 generation pups in either BMN 270-19-008 study or BMN 270-19-033 study. There was no case of DNA vertical transmission from F0 male to F1 pups in both studies. The applicant calculated the probability that the risk of germline transmission is below 5%. When combining the results from both studies, there is 99.2% confidence that the risk of germline transmission is below 5%.

BMN 270 is an ATMP. Consequently, traditional pharmacokinetic analyses have not been conducted to investigate the classical aspects of absorption, metabolism or excretion of BMN 270. However, biodistribution of BMN 270 in liver as well as other tissues and organs has been assessed in the CD1 normal mice and in Rag2-/- x FVIII-/- double knockout mice.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

The nonclinical toxicology programme was designed to support a single IV infusion in patients with haemophilia A. As the clinical schedule foresees one unique injection of BMN 270, all non-clinical studies supporting the safety of BMN 270 are single-dose toxicity studies conducted by the IV route, in male animals, in different animal species ($Rag2^{-/-}$ mice, $Rag2^{-/-}$ x $FVIII^{-/-}$ mice, CD1 mice, monkeys), which is acceptable.

- General toxicity:

First, the initial safety assessment of BMN 270 was performed in studies intended to establish proof-of-concept in $Rag2^{-/-}$ x $FVIII^{-/-}$ mice which served as an animal model of haemophilia A (study BMN 270-14-076) or in $Rag2^{-/-}$ mice (study BMN 270-16-049). These pharmacodynamic studies in $Rag2^{-/-}$ mice or in $Rag2^{-/-}$ x $FVIII^{-/-}$ mice included safety assessments endpoints including clinical observations, clinical pathology and histology.

In **Study BMN 270-14-076**, $Rag2^{-/-}$ x $FVIII^{-/-}$ male mice were injected with escalating doses of BMN 270 (from 2.10^{12} to 2.10^{14} vg/kg). For the lower doses groups (groups 2, 3, and 4), hFVIII-SQ protein was not detectable in plasma. FVIII activity increases (in % of normal human plasma) were significant only in high dose groups (groups 5 and 6). Noteworthy findings included a mild interstitial, periglomerular, and glomerular kidney inflammation occurring in 9 treated animals across all groups. The applicant further justified that i) these findings lacked a clear dose-response, ii) the severity of the findings was generally minimal to mild and iii) these findings neither had an impact on the study endpoints nor any adverse impact on the overall welfare of the mice. The toxicological relevance of these findings was thus unknown. As no similar findings were observed in other mouse and NHP studies, these findings appeared incidental and refined to study BMN 270-14-076.

Also, lymphoma was present in two animals treated with the lower doses of BMN 270 (2.4E10 and 1.7E11 vg/kg). They were considered related to the immunologically compromised nature of these $Rag2^{-/-}$ x $FVIII^{-/-}$ mice (which lack mature B and T cells, making them highly susceptible to infections and spontaneous development of malignancies such as lymphoma and acute leukaemia). Along these lines, the observed haematopoietic malignancies in study BMN 270-14-076 could be related to this immunodeficiency; as lymphomas are among the most common tumors in many strains and stocks of

mice (C57BL/6 mice develop 10-50% incidences of lymphomas in aged mice). To note, no lymphomas were observed in the same strain of mice treated at the higher dose levels of BMN 270.

The aim of **Study BMN 270-16-049** was to compare the pharmacodynamics and liver distribution of BMN 270 test article produced either with process C (phase I/II material) or with process D (phase III material) in the $Rag2^{-/-}$ mice. As already observed in previous studies, some animals treated at the dose of 6.10^{12} vg/kg (between 3/10 to 5/10 animals) did not respond to BMN 270 treatment, regarding hFVIII-SQ protein expression. After 5 weeks of observation, there were no test-article related clinical observations, no test-article related changes in body weight or food consumption through the entire study. The macroscopic necropsy examination did not reveal any remarkable findings. As histopathology examination was not conducted on this study, it was not possible to check if similar findings that those noted in previous study BMN 270-14-076 (mild interstitial, periglomerular, and glomerular inflammation, lymphoma) were also present in these groups of $Rag2^{-/-}$ Mice. In terms of safety assessment, this study was of limited interest.

Then, two GLP single-dose studies were conducted in normal CD1 mice with observation periods of up to 26 weeks, and with doses ranging from 6E12 to 2E14 vg/kg. They both addressed the biodistribution and potential toxicity of BMN 270. These studies are considered pivotal. The first GLP toxicity study (**Study BMN 270-14-030**) conducted in CD1 normal male mice used a batch of BMN 270 produced according to process B, which far diverges from process D and D' used in phase III clinical trials and commercial process. This is a limitation of the study.

For animals dosed with 6.10^{12} vg/kg, there was no quantifiable hFVIII-SQ protein, nor discernible increase in FVIII activity from murine endogenous levels. However, protein expression was quantifiable in animals treated at 6.10^{13} vg/kg. BMN 270 was well tolerated in normal CD1 male mice, as there was no mortality, no adverse clinical observations, body weight changes, gross lesions and histopathology observations or toxicologically important differences in clinical pathology parameters. However, as this study used a batch of BMN 270 drug product manufactured according to process B, which is an early research/development process, it appears that the test article administered to CD1 mice cannot be considered as representative of the clinical formulation produced according to commercial process CPD'. This is a limitation of the study; consequently, safety data gained in the course of this study can only be considered as supportive.

However, in **Study BMN 270-16-045**, realised in the same strain of mice (CD1 normal male mice), significant toxicological findings were noted. Eight treated mice died in the course of the study (3 in the 6.10^{13} vg/kg group, 5 in the 2.10^{14} vg/kg group). The histopathology examination revealed myocardial necrosis, haemorrhage, vascular and perivascular necrosis, fibrosis, and inflammation in the heart. Microscopic observations also included fibrosis, haemorrhage and macrophage infiltrates in the epididymis, and oedema, haemorrhage and mesothelial hypertrophy in the lungs. Since many lesions were associated with haemorrhages, and because of the temporal relationship, these overall toxicological findings suggest that they were the clinical result of antibody formation against the expressed human FVIII-SQ, which is heterologous in mice. These anti-hFVIII antibodies cross-reacted with the murine endogenous FVIII, leading to a loss of endogenous FVIII activity, a bleeding phenotype, and subsequent visceral lesions (in heart, lungs and epididymis) associated with haemorrhages. To rule out a possible contribution of BMN 270 in the occurrence of such findings, the applicant further emphasised that there were no instances of mortality or morbidity in investigational studies conducted with $Rag2^{-/-}$ mice (another strain of mice) which is not capable to elicit a humoral immune response, that were dosed as high as 6E13 vg/kg and lasted up to 24 weeks post-dose (BMN 270-14-075, BMN 270-16-049, BMN 270-17-035). This observation supports the hypothesis that the histopathological lesions found in Study BMN 270-16-045 were due to the formation of anti-hFVIII antibody responses, as $Rag2^{-/-}$ mice lack a functional adaptive immune response.

The applicant also specified that the above findings should be placed in the context of expressing hFVIII-SQ in CD1 mice, which have an intact coagulation system and a fully functional immune system. The toxicological effects attributable to FVIII antibodies identified in this study are likely the result of an immune response specific to the heterologous human FVIII-SQ protein in mice and are not expected to translate to HA patients in the clinic.

Importantly, data from patients that were dosed with BMN 270 (clinical studies 270-201 and 270-301) have been accumulating for the past 4.5 years (219 weeks), including patients dosed with Process D, and no bleed related serious adverse effects or formation of antibodies against FVIII have been observed. No inhibitor responses have been detected in any of the patients. This is consistent with the argument that the findings in Study BMN 270-16-045 are immune-mediated and species-specific.

Secondly, additional safety assessments were performed in single-dose monkey studies designed to investigate the PD of BMN 270, to compare different capsid types, to compare methods of vector production, to evaluate PD effects due to pre-existing anti-AAV5 immunity and to evaluate the PD effects of steroids.

The objective of **Study BMN 270-13-031** was to select the AAV-vector capsid for Factor VIII gene therapy, and to assess the relative activity of the two capsids (AAV5.2 FVIII-SQ and AAV8.2 FVIII-SQ) with FVIII.SQ transgene, over 8 weeks when given as a single slow bolus IV administration (2E12 or 2E13 vg/kg) to cynomolgus monkeys. BMN 270 was rather well tolerated; specifically, the lack of adverse effects on clinical pathology parameters suggested that neither vector overtly affected liver function. There were no adverse effects on clinical signs (only bruising), food consumption, body weights, clinical pathology or gross pathological or organ weight effects observed up to 8 weeks following a single IV dose. Both vectors capsids appeared well tolerated. But all monkeys, independent of the dose, developed anti-AAV5 antibodies.

Study BMN 270-14-014 determined activity of BMN 270 drug product when manufactured either in the baculovirus system (baculo-270) or in 293 cells (293-270). The only BMN 270 related finding was a prolonged activated partial thromboplastin time (APTT) occurring in four animals, indicating that they were less able to coagulate, though interspecies cross reactivity of anti-hFVIII-SQ antibody with monkey FVIII has not been assessed by the applicant. There were no BMN 270-related changes in liver clinical chemistry, indicating lack of overt effects on liver function or other notable toxicity at doses up to 2E13 vg/kg. All AAV5-FVIII-SQ-Proto1 treated animals developed antibodies against AAV5 by 4 weeks post dosing.

The aim of **Study BMN 270-14-062** was to assess the relative activity of BMN 270 in two different species of nonhuman primates (cynomolgus or rhesus monkeys) over 6 weeks when administered as a single IV injection. All cynomolgus and rhesus monkeys were prescreened for anti-AAV5 antibodies and AAV5 transduction inhibition activities prior to assignment to the study.

All animals survived to the end of the study, except one rhesus monkey (Animal No. 3001) administered 6E12 vg/kg, which had to be euthanised on Day 14 due to decreased body weight, and apparent morbidity. Body weight loss was already observed before BMN 270 injection, beginning Week -1 (pre-dose) through Week 1. Microscopic correlates in the colon included mild neutrophilic inflammation, epithelial degeneration (characterised by epithelial attenuation, segmental loss of epithelium, and decreased goblet cells), and moderate haemorrhage. Findings in the colon were considered the primary cause of the animal's moribund condition and contributed to many of the clinical findings (e.g. decreased activity, liquid feces, dehydration). Because of the progressive body weight loss prior to administration of BMN 270 (Study Week -1 through Study Week 1), microscopic findings suggesting an ongoing condition (thymic changes), and the absence of similar clinical or macroscopic findings in other dosed animals, the moribund condition observed in Animal No. 3001 was considered unrelated to the administration of BMN 270. It is however noticed that ASAT, ALAT, ALP

and LDH were increased by day 8; concomitantly with an increase in PT and APTT. The applicant specified that elevations in hepatic enzymes (ALAT, ASAT, LDH and ALP) correlated with concurrent clinical signs of gastrointestinal tract abnormalities and appeared most likely related to the enteropathy. Histopathologic examination of the liver from the same animal (#3001) on Study Day 14 (i.e. 1 week after the exhibiting the clinical signs of gastrointestinal dysfunction) revealed no inflammation/hepatitis. Microscopically, the liver had fatty change (lipid vacuoles) and no other findings consistent with normal hepatic transaminases (ALAT/ASAT). Overall, it was estimated that these arguments can rule out the possibility of a toxic effect, due to the BMN 270 infusion.

An addendum was included in study BMN 270-14-062 to include complementary brain histopathology analysis: microscopic evaluations of brain, sciatic nerve, and spinal cord (including dorsal root ganglion) were performed at day 43 (euthanasia) on retained samples. For 8 animals (Rhesus or Cynomolgus) treated at 6.10^{12} or 2.10^{13} vg/Kg, there was no evidence for abnormalities in the dorsal root ganglia, nor in the spinal cord area, at the time of euthanasia. However, the pathology report indicates that one animal treated at 2.10^{13} vg/Kg displayed a minimal mononuclear cellular perivascular infiltration in brain**.**

The applicant stressed that the observed pathology finding (ie minimal mononuclear and perivascular cellular infiltration) occurred in brain parenchyma in only a unique animal (animal # 4001) in the course of study BMN 270-14-062. The applicant referred then to several references from literature (Sato, 2012; Chamanza, 2010, Butt, 2015) stating that lymphoplasmacytic perivascular (mononuclear) infiltration is commonly seen in general organs of NHP, and appears as a background finding, that occurs incidentally in macaques.

Besides, no other findings were observed in the examined neural tissues, particularly in the dorsal root ganglia, which is a site of tropism of rAAV vectors. Concluding, this single incidence of perivascular mononuclear infiltrate with no damage to surrounding tissue or evidence of test-article related inflammation is considered as an incidental finding by the applicant.

No other animals showed any BMN 270-related adverse effects as indicated by lack of overt clinical signs, or changes in food consumption, body weights, clinical pathology, gross necropsy observations, or organ weights.

The objective of **study BMN 270-16-021** was to determine the comparative PD of BMN 270, when administered as a single IV bolus injection to cynomolgus monkeys with varying anti-AAV5 total antibody titers (TAb) and evidence of transduction inhibition (TI) as assessed during screening and at baseline. There were no test article-related changes in clinical observations, body weights, food consumption, coagulation and clinical chemistry parameters, gross necropsy findings, or histopathology in the liver. Major clinical signs included localised abrasions, bruises, and rectal prolapse.

However, a significant decrease in neutrophils was noted in all groups from Day -6 to Day 56, but not commented by the applicant. A BMN 270-related mild increase of lymphocytes occurred in Group 4 (TAb+ and TI titers of 6-100) at Day 56, and was statistically significant. The mean lymphocytes increase was 1.5x as compared to Group 1 (TAb- and TI-). The applicant was requested to comment on decrease in neutrophils as well as increase in lymphocytes. At the first round of the precedent submission (Day 80), the applicant acknowledged the significant decrease in neutrophil numbers as well as the important increase in lymphocyte numbers, observed at day 56, amongst animal groups. No clear relation was apparent between these alterations (neutrophils and lymphocytes) and Tabs/TI titers. The applicant has no formal explanations on the causes/origins of these alterations; besides no control group (vehicle treated) was included in the study. Of note, at the clinical level, decreases in neutrophils and lymphocyte counts have been observed in some subjects. These changes were transient, did not require intervention and were not considered clinically significant by the investigator. The issue was considered as solved.

2.5.4.2. Repeat dose toxicity

No repeat-dose toxicity studies were performed because BMN 270 is a single-dose administration gene

2.5.4.3. Genotoxicity

In terms of genotoxicity, no studies were performed with BMN 270. The applicant provided literature references (Chandler, 2015); (Li, 2011); (Niemeyer, 2009); (Schnepp, 2006) and the Draft FDA Guidance for Industry on Human Gene Therapy for Haemophilia (2018), which support the position that there is very little risk of either genotoxicity or carcinogenicity associated with AAV5 vector products or recombinant FVIII proteins. The applicant also argued that the integration frequency of engineered AAV vectors is orders of magnitude lower than the spontaneous rate of mutation for human genomes (Cole, 1994), so the likelihood of insertional mutagenesis by AAV vectors is very low. However, recent publications/abstracts on the use of rAAV vectors in haemophilia dogs raised the problematic of integration of rAAV vectors inside host cell genome. Indeed, in a recent communication of Giang N. Nguyen, BS et al. (2019), HA dogs were treated with AAV-canine FVIII. ISA (integration site analysis) was performed on liver samples, and IS analysis revealed >2,000 unique AAV integration events. There was also a correlation between the DNA copy number and the number of integration events detected. Integrations events were distributed across the canine genome. Clonal expansions were also observed with integration near genes previously associated with growth control and transformation in humans. Importantly, the authors questioned consequently the observed increase in FVIII expression, which could be associated with the clonal expansions detected.

Recent publications/abstracts on the use of rAAV vectors have again raised safety concerns in terms of insertional mutagenesis and carcinogenesis (see discussion in overview). The applicant was requested to provide an empirical risk assessment on insertional mutagenesis. Data from integration site analysis was expected (obtained on archived monkey liver tissue samples, or on retained liver samples from CD1 mice or NHP, treated with doses superior or equal to 6.10^{13} vg/kg). In response to the question, the applicant provided results from **Study BMN 270-20-013**, whose objective was to conduct integration site analysis using Target Enrichment Sequencing (TES) on 12 cynomolgus monkey liver samples transduced with BMN 270.

The Target Enrichment Sequencing (TES) method was used to identify BMN 270 vector integration sites in non-human primate liver samples after administration of a single dose of BMN 270 or vehicle control. Liver samples from the left medial lobe of 18 cynomolgus monkeys administered a single dose of BMN 270 (n=12) or vehicle control (n=6) via slow bolus intravenous injection under Study BMN 270-16-046 (doses: 2.10^{13} and 6.10^{13} vg/Kg) were analyzed. 62,263,872 sequences were analyzed: this gave 9,227 insertions, most other sequences being vector-vector sequences. An average of $1{,}55.10^{-3}$ insertion/cell frequency was estimated. Most integrations were unique, which suggests an absence of common insertion profile, and an absence of clonal expansion. Common integration sites (CIS) were defined as being common integration in a 50kb window region. Cancer genes were identified when integration occurred in a window of +/- 100kb region.

In a study in which dogs with haemophilia A were treated with AAV8/9 gene therapy (containing the canine Factor VIII gene), analysis of liver specimens 10 years post-dose identified clonal populations of FVIII-producing cells harbouring vector DNA integrations in 5/6 dogs, whereby 44% of the integrations were located near genes involved in cell growth (Nguyen et al., 2021, DOI: 10.5281/zenodo.3666122). Even though no tumours were identified, these findings likely indicate pre-stages of malignancy.

The applicant also communicated during the procedure that in another AAV5-based GTMP programme (BMN307, an investigational AAV5-phenylalanine hydroxylase gene therapy), 6/7 male mice that received BMN307 at 2*10E14 vg/kg (highest dose group) had tumours on liver necropsy 52 weeks

after dosing with evidence of integration of BMN307 into the genome. Of note, no macroscopic lesions were observed in livers of any mice taken down at 24 weeks

The results of these dog and mice studies together with the abundant integration of BMN 270 in the vicinity of cancer genes in cynomolgus monkeys demonstrates that insertional mutagenesis and ultimately insertional oncogenesis is an inherent risk of i.v.-administered AAV therapies such as BMN 270 in laboratory animals.

As already mentioned before, the average IS/cell in all 12 BMN 270 treated monkeys was 1.55E-03, and the average unique IS/vg in the different samples was 6.10E-05 IS/vg. Using these numbers, the total number of integrations in patient's livers upon administration of BMN 270 can be estimated by the following two approaches:

1.) The planned posology of BMN 270 in patients is the following (SmPC): 6*10E13 vg/kg body weight, equalling 4.2*10E15 vg in total for a 70 kg heavy patient. Considering the average of 6.1*10E-5 IS/vg established in cynomolgus monkeys, one would obtain 2.56*10E11 integrations after administration of BMN 270 to a 70 kg patient (4.2*10E15 * 6.1*10E-5). It should however be noted that only a fraction of the administered viral genomes will transduce hepatocytes. In the cynomolgus monkey study BMN 270-16-046, this fraction can be estimated to be only 0.16%: By assuming that the cynomolgus liver has the same hepatocellularity as the human liver (139*10E6/g liver tissue, https://pubmed.ncbi.nlm.nih.gov/16930941/), that the cynomolgus liver weighs 70g, that 24 vg copies were found per hepatocyte, and by considering that 1.5*10E14 vg's were administered to cynomolgus monkeys weighing 2.5 kg (2.5 * 6*10^13), the total vg's in the monkey liver were 2.34*10E11 (70*24*139*10E6), giving a fraction of 0.0016 (0.16%) between the administered vg's and the vg's in the liver (2.34*10E11 / 1.5*10E14). By including this fraction (0.16%) in the extrapolation of the integration load in patients, the amount of integrated vg's in human hepatocytes can be estimated to be **3.99*10E8** (2.56*10E11 * 0.0016).

2.) Assuming that also in humans approximately 1.55E-03 IS/hepatocyte applies, approximately **3.88*10E8** hepatocytes of patients could be affected by vector genome integration (1.55E-03*139*10E6*1800), when assuming that the human liver weighs 1.8 kg and has 139*10E6 hepatocytes per gram of liver tissues (https://pubmed.ncbi.nlm.nih.gov/16930941/). This number is in the same range as the number estimated before.

Considering that up to 10% of the ISs were located in the vicinity of cancer genes in liver specimens of the cynomolgus monkey study BMN 270-16-046, roughly 40 million hepatocytic integration events close to cancer genes could occur in patients after having received BMN 270 (when referring to the estimates of total hepatocyte transgene integrations in patients extrapolated before). As a significant fraction of the detected integration events in cynomolgus livers in study BMN 270-20-013 occurred in the vicinity of cancer genes, it appears likely that treatment with BMN 270 might inflict insertional mutagenesis and ultimately insertional oncogenesis. Even though the dimension of this risk in patients remains unclear, non-clinical studies of other AAV-based therapies demonstrate that AAV-transgene integration can potentially manifest in tumorigenesis.

2.5.4.4. Carcinogenicity

As regards carcinogenicity: no carcinogenicity studies were performed with BMN 270. The applicant argued that the risk for carcinogenic potential of the expressed transgene product, hFVIII-SQ, is anticipated to be low because it lacks immunomodulatory or cellular-proliferation activity.

Considering the AAV vector by itself, AAV5 vectors (including BMN 270), are replication defective and contain no viral genes. AAV vectors persist for prolonged periods as episomal structures and integrated copies of AAV vector genomes are rare. There are observations of liver integration of AAV genomes in

various mouse models. Relevance to human risk is confounded by inter-study variability, vector construct dependencies, murine specific integration sites and murine specific physiology (higher ploidy than typical liver diploid cells, contribution of disease progression, mouse strain specific spontaneous tumour formation) (Zhong, 2013); (Chandler, 2015); (Bell, 2006).

Extensive studies with AAV2 and AAV1 vectors in rodents (Schnepp, 2003); (Inagaki, 2008); (Li, 2011), rabbits (Schnepp, 2006), nonhuman primates (Nowrouzi, 2012) and in human subjects who were administered Glybera (an approved gene therapy product) (Kaeppel, 2013) lead to the estimation that the integration frequency of AAV vectors is several orders of magnitude lower than the spontaneous rate of mutation for human genomes (Cole, 1994) so that the likelihood of insertional mutagenesis by AAV vectors is very low. The applicant was requested to discuss more in depth the risk associated with carcinogenicity when using BMN 270. Indeed, in a recent publication from Zucman-Rossi et al., Nature Genetics, 2015, the authors found clonal integration of AAV2 in 11 of 193 hepatocellular carcinomas (HCCs). AAV2 integrations occurred in known cancer driver genes, namely CCNA2 (cyclin A2: four cases), TERT (telomerase reverse transcriptase: one case), CCNE1 (cyclin E1: three cases), TNFSF10 (tumour necrosis factor superfamily member 10: two cases) and KMT2B (lysine-specific methyltransferase 2B; one case), leading to overexpression of the target genes, and consequently, oncogenicity events. The authors concluded that AAV2 viruses were DNA viruses associated with oncogenic insertional mutagenesis potential in human HCC.

The applicant specified that, to date, more than 200 clinical trials have been conducted with rAAV mediated gene therapy (Ginn, 2018) with no reports of rAAV-mediated genotoxicity. No cases of AAV-mediated insertional oncogenesis have been reported in humans. Vector DNA quantities show a decline over time without kinetics consistent with clonal expansion. As of 12 Dec 2019, 151 subjects have received BMN 270 followed for up to 4.2 years (219 weeks) with no reports of malignancies across any clinical trials. In addition, FVIII levels are steady with no evidence of increasing FVIII levels otherwise associated with clonal expansion reported in dogs (Nguyen et al., 2019). The risk of AAV-mediated oncogenesis in humans is therefore considered theoretical, with no genotoxic events reported to date. Based on these considerations, the applicant estimated that the oncogenic and tumorigenic potential of AAV vectors including BMN 270 is currently considered to be low or negligible. Results presented in the recently conducted study BMN 270-20-013 confirm that some integration events can occur in the cell genome, but with an average integration frequency of 1.55E-03 IS/cell (across all 12 animals in groups 3-5), demonstrating that integration of the BMN 270 vector is a rare event in liver cells, in accordance with published literature of other AAV vectors.

However, recently, the applicant shared a communication with EMA about an ongoing BioMarin gene therapy programme evaluating BMN307, an investigational rAAV5 vector encoding the-phenylalanine hydroxylase (PAH) in the treatment of phenylketonuria. Biomarin informed health authorities about interim safety findings from a pre-clinical study. This non-clinical study was conducted in mice bearing two germline mutations (one mutation for inactivation of the PAH missing in PKU, and the second rendering the animals immunodeficient). Of 63 animals treated, six of seven animals had histologically confirmed tumors on liver necropsy 52 weeks after dosing with evidence for integration of portions of the BMN307 AAV vector into the host genome. Five of the animals taken down at 52 weeks had adenomas and one had a hepatocellular carcinoma (HCC).

2.5.4.5. Reproductive and developmental toxicity

As concerns fertility, dedicated fertility studies/embryofoetal studies have not been performed; the applicant argued that males comprise the majority (>99%) of the patient population to be treated with BMN 270 (Note: women are not excluded from the targeted population according to the current

SmPC). However, general toxicity assessment in studies BMN 270-14-030 and BMN 270-16-045 revealed some effects on testes (tubular degeneration). In study BMN 270-16-045, tubular degeneration occurred only in treated males, and not in controls, at the three time points assessed (day 28/29, Day 91, day 182). This finding was challenged. In its response, the applicant argued that tubular degeneration in testes was essentially observed in study BMN 270-16-045, where 2 mice dosed with 6.1013 vg/kg and 2 mice dosed with 2.1014 vg/Kg experienced this type of finding, whereas no such finding was observed in control mice. The applicant argued 1) that it was most likely an incidental (background) finding, as similar observations were made in others rodent toxicity studies (Creasy, 2012; Foley 2001); 2) that there was no dose-response regarding this adverse effect, and 3) the incidence of the finding was low. This issue was not further pursued.

In a second germline transmission study (see PK section, Study BMN 270-19-033), safety parameters on F0 animals and F1 pups generation were followed. F0 male mice were treated (6.1013 vg/kg) and mated with untreated F0 females. The F1 pups were assessed for presence of vector DNA in their liver. No BMN 270 DNA was detected in the liver of any F1 generation animal. In terms of toxicity, only gross necropsy was performed on F1 pups, without histopathology examination. The applicant concluded that there were no F1 generation clinical observations or differences in pup body weights related to the administration of BMN 270 or necropsy observations in any of the F1 generation pups. However, 28 F1 generation early deaths occurred on study: 12 F1 generation issues from F0 control parental animals and 16 F1 generation issued from F0 treated parental animals. The applicant indicates that the cause of death/unscheduled euthanasia could not be determined, but based on tissues evaluated, early deaths were considered likely unrelated to BMN 270 administration based on the lack of BMN 270-related clinical observations and gross and microscopic findings, and there were also early deaths in the control group.

- Studies in juvenile animals: A GLP juvenile mouse study was completed to assess the pharmacodynamics and potential toxicity of BMN 270 in Rag2-/- mice of various ages. This study was intended to support future dosing of adolescents and children with BMN 270. Nonetheless, the current MA application seeks approval for treatment in adult patients only. Thus, BMN 270-18-012 study is not of primary interest for the primary indication. As well, this study will be thoroughly reviewed at the time of the submission of the extension of indication in the paediatric population.

2.5.4.6. Local tolerance

No local tolerance studies were performed because BMN 270 is administered intravenously. In addition, there were no observations of injection-site reactions in any of the nonclinical studies performed. This is endorsed considering the IV route of administration for BMN 270 injection.

2.5.4.7. Other toxicity studies

Other toxicity studies have been conducted, in particular to assess the effect of concomitant chronic steroid treatment (Study BMN 270-17-002). The first objective of this study was to determine if chronic steroid treatment following BMN 270 treatment in wild type mice could impact hFVIII-SQ expression. The second objective was to determine whether liver enzymes were altered following BMN 270 treatment with or without chronic steroid treatment in wild type mice. The following conclusions may be drawn: i) treatment with prednisolone for 3 or 12 weeks did not modulate the levels of hFVIII-SQ protein or FVIII activity in plasma, at either vector dose (2.1013 vg and 6.1013 vg/kg), ii) the percent of hepatocytes staining positive for FVIII by IHC was not different between steroid- and water-treated mice given BMN 270 at 6e13 vg/kg and iii) there were no statistically significant increases in the mean plasma level of either aspartate aminotransferase (AST) or alanine aminotransferase (ALT) in response to treatment with BMN 270 or prednisolone. In conclusion, daily treatment with

immunosuppressive doses of prednisolone for 3 or 12 weeks, beginning one week after administration of BMN 270 at either 2e13 or 6E13 vg/kg, did not affect hFVIII-SQ expression.

2.5.4.8. Immunogenicity

Immunogenicity to the vector capsid was assessed in both species, and this revealed that, independent of the dose, one unique injection of BMN 270 vector elicited anti-AAV5 antibodies. In the same way, animals elicited immune humoral responses against hFVIII-SQ, leading to loss of FVIII activity, and in some cases with coagulopathy associated with bleeding phenotype (normal CD1 mice). But, according to the applicant, no neutralizing immune responses were reported up to date in clinical studies.

2.5.5. Ecotoxicity/environmental risk assessment

AAV5-hFVIII-SQ is derived from naturally occurring AAV of serotype 5, which has been genetically engineered to vehicle a therapeutic gene (hFVIII optimised cDNA) and to render it replication-incompetent. These modifications have not altered the host range, or tropism of the vector, with a preferential, while not exclusive, gene transfer to the liver.

Due to the defective autonomous replication capacities of the vector, to the favoured liver tropism, and based on past experience accumulated by the use in diverse clinical protocols of AAV-vectors, there have been no reported environmental effects identified that could be caused by the release of AAV5-hFVIII-SQ.

The shedding of the virus appears time-limited, while some patients seems to release vector DNA after several weeks. However, this shedding, might be not linked to infectivity is extremely unlikely to lead to infection of non-target human, animal or plant cells.

The applicant addressed the potential horizontal transmission regarding the patients with a prolonged semen shedding. Based on the time necessary to obtain the first of the three consecutive negative semen samples of 131 patients from potentially infectious particles (encapsided particles) of 3.00 weeks [min:0.429, max: 12.1] with a maximum time of 12,1 weeks, the proposed 6-month contraceptive measures for males treated and their female partners of child bearing potential are endorsed without additional requirement.

Considering that the vector is replication-incompetent, that AAVs are not pathogenic, and that the transgene is not harmful, it can be agreed that the risk to the environment is negligible.

2.5.6. Discussion on the non-clinical aspects

From a pharmacology point of view, it is acknowledged that an adequate set of experiments have been conducted in different animals species, eg normal C57BL/6J and Crl:CD1(ICR) mice, Rag2-/- mice, Rag2-/- x FVIII-/- mice, and Rhesus and Cynomolgus monkeys. Proof of concept has been determined in the Rag2-/- x FVIII-/- mice in the course of Study BMN 270-14-014.

In mice, first studies conducted in the Rag2-/-xFVIII-/- mice with suboptimal doses of BMN 270 (6E11 vg/kg and 2E12 vg/kg) revealed that even if vector transduction occurred in liver, there was no subsequent hFVIII-SQ protein protein expression by the liver. Further doses ranging studies performed in the Rag2-/-xFVIII-/- mice, but with higher doses (2E13 vg/kg and 2E14 vg/kg), allowed to assume that a threshold of dose of 2.1013 vg/kg is necessary to detect and achieve (a minimum of) quantifiable level of hFVIII-SQ expression, and increase in FVIII activity (% of human normal). A large increase was noted for hFVIII-SQ expression and FVIII activity when the BMN 270 dose increased from 2.1013 to 2.1014 vg/kg. Considering the claim for a sustained pharmacological effect, further results suggested that a threshold of dose of 6.1013 vg/kg was necessary to achieve in the Rag2-/- x FVIII-/-

mice, a sustained pharmacological effect lasting for a period of 13 weeks.

However, in study BMN 270-14-030, conducted in the normal CD1 mice, even at the highest dose of 6.1013 vg/kg, results at day 90 appeared quite disparate/irregular among animals. One animal out of five was BLQ for hFVIII-SQ expression, not responding to treatment. In the same way, at 6.1013 vg/kg, on Day 90, 3 of 5 mice dosed with BMN 270 had levels of FVIII activity beyond what was expected from endogenous murine FVIII, demonstrating an important disparity/heterogeneity in FVIII activity results. The same widespread results were also observed in study BMN 270-16-045, using higher BMN 270 doses (6E13 vg/kg and 2E14 vg/kg). Considering the important disparity results in hFVIII-SQ expression and FVIII activity in mice, this observation raises the question/notion of responders versus non-responders individuals to BMN 270 treatment.

In monkey studies, animals were injected with 2E12 vg/kg/2E13 vg/kg or 6E13 vg/kg. Plasma hFVIII-SQ levels were obtained in animals, but were transient over time: hFVIII-SQ levels increased to reach a Cmax by 4/5 weeks, and declined thereafter, to reach insignificant levels by weeks 5 to 8. BMN 270 vectors elicited anti-AAV5 antibodies. Of note, a pharmacological sustained effect (ie a plateau phase for hFVIII-SQ plasmatic concentrations) was not demonstrated over time, particularly in the 4 monkeys dosed at 6.1013 vg/Kg.

A study conducted in juvenile animals was completed. The objective of this GLP study was to determine the future dosing in children and adolescents, by demonstrating the safety of BMN 270 following a single intravenous injection to RAG2-/- x FVIII-/- double knockout (DKO) male mice at different ages and to determine expression profile of hFVIII-SQ as a function of age and post-natal development. It was noticed that the applicant did not provide any general synthesis nor discuss the results obtained in terms of hFVIII-SQ expression and DNA and RNA persistence over time, according to liver growth as a function of age and post-natal development.

Of note while currently out of the scope of the procedure focused on adults, during the review process the applicant outlined that the question of the optimal age for child treatment with BMN 270 appears complex, as the maturational rate of mice does not linearly correlate with humans. Indeed, it appears that mice dosed at younger ages (ie 1 or 2 weeks) have lower plasma hFVIII-SQ protein, hFVIII-SQ DNA and FVIII activity compared with those dosed at older ages, ie at 6 or 8 weeks. According to the applicant, the optimal age to administer AAV5-FVIIISQ in mice would be 4 weeks or older as these mice had higher plasma hFVIII-SQ concentration and FVIII activity when dosed at this time. However, data gained in NHP studies showed different relationships between dose-timing and transduction patterns than those observed in mice. Overall, the applicant announced that it will work with the PDCO at the time of the submission of an extension of indication in children. For the time being, the application is only seek in adults.

From a **pharmacokinetic** point of view, results showed that BMN 270 genome distributes widely many organs and tissues. This observation is substantiated by data from studies BMN 270-14-030 and BMN 270-16-045. At the first sampling time point (day 28), spleen and liver appeared as the most distributed organs. In terms of clearance, vector DNA levels were generally seen to decrease over time within each tissue/organ, from day 28 to day 91 to day 182. But it is noteworthy to mention that all organs/tissues remained positive for DNA detection at the latest study time point (day 182). Of note, at the dose of 6.1013 vg/kg, testes were still positive in hFVIII-SQ DNA detection at the last study time point (1996 vg/mg of tissue at day 182).

As concern the finding on increasing DNA concentrations in brain between 4 weeks (day 28) and 12 weeks (day 91) post administration of a 6.1013 vg/kg dose, the applicant provided reassuring data showing a partial and gradual elimination of DNA genomes from brain tissue over time, allowing to think that an elimination kinetics is in place in brain tissue after one unique BMN 270 injection. Of note, the applicant did not give an estimation of the delay necessary for a total clearance from brain,

although requested; this could have been provided. As no major BMN 270 related safety concerns have been reported in CNS at the clinical safety level, this issue is no longer pursued.

From a **toxicological** point of view, further investigations were conducted on retained NHP brain samples, with complementary brain histopathology analysis: microscopic evaluations of brain, sciatic nerve, and spinal cord (including dorsal root ganglion). The absence of abnormalities in the dorsal root ganglia and in the spinal cord area was particularly noted.

Overall, two germline transmission studies were performed. In both studies, the applicant transmitted mean hFVIII-SQ DNA values obtained in testes from F0 male mice, harvested on Day 50. Given the DNA levels present in the sperm of F0 male animals at the time of mating, and in light of concentrations observed in the sperm of male patients after a 6 month contraception period which is foreseen in clinics, the risk of transmission to the female partner cannot be excluded. Therefore, as reflected in the SmPC/PL both male treated and his woman partner of child bearing potential should have contraceptive measures.

It was noticed that sperm/semen from F0 parental animals were not tested for hFVIII-SQ DNA detection. This absence of analysis (cell fractioning) on the sperm/semen of F0 animals appears detrimental, as more information was needed to know which species/forms of the BMN270 vector (infectious/noninfectious, heavy particles, intermediate particles or free DNA) localize in which compartment of the semen/sperm. Biomarin specified that results from clinical study BMN270-301 revealed that potentially infectious particles (ie reflecting encapsidated vector DNA) were detectable in semen from 131 of the 133 subjects (98.5%) subjects. 131 patients had their first sperm negative sample in a maximum delay of 12 weeks. Having in mind the duration of viral shedding and the spermatogenesis, a 6 months contraceptive measures is recommended for woman partner of man treated.

Finally a reprotoxicity study is ongoing that might inform on the risk for the newborn if Roctavian is used in women of childbirth potential (WOCBP). The use of the product in WOCBP is currently not recommended pending those data, with explicit statements in section 4.6 of the SmPC for alerting women on the reasons for this not recommended use, i.e. it could be harmful for the new-born child (theoretical risk of viral vector integration in foetal cells through vertical transmission). Moreover, no data are available to recommend a specific duration of contraceptive measures in women of childbearing potential.

Regarding **genotoxicity**, the applicant conducted an integration site analysis study, using Target Enrichment Sequencing (TES) on 12 cynomolgus monkey liver samples transduced with BMN 270. Livers were collected from NHP dosed either at 2.1013 vg/Kg or 6.1013 vg/Kg. Livers were collected at week 13 or week 26 post-injection. Some questions were raised related to the design of the study, but findings indicate an average of 1,55.10-3 insertion/cell frequency. Most integrations were unique, which suggests an absence of common insertion profile, and an absence of clonal expansion. Cancer genes integrations were rare (around 6% of the genes), and there were no preference for specific cancer genes. As such, such a feature has to be considered on the long term: Indeed, while AAV are not expected to integrate their genome in host cells at high frequency, all integration events could contribute to transformation.

As mentioned above, the applicant explains that, a very small fraction of the BMN 270 vector, on average less than 0,1%, was found to have integrated rAAV into chromosomal DNA. While 0,1% of integration appears low, in a whole liver it corresponds to 10^7 integrations (with a number of 10^{10} hepatocytes in an adult liver).

The translatability and clinical relevance to humans need a longer period of observation to validate that aspect of the safety profile for rAAV. The applicant underlines that insertional tumorigenesis remains a

theoretical risk to date, when considering the treatment of haemophilia patients with rAAV vectors. However, cautious is warranted given the late breaking event reported of acinic cell parotid carcinoma (see clinical safety part) reported in a male patient approximately 5 years after having been dosed with Roctavian 6E13vg/kg in study 270-201 study.

Animal data must be split between mouse data and dog data, as insertional tumorigenesis linked to administration of an AAV vector has only been observed in nonclinical mouse studies (and not in larger animals or humans).

In mice, integration associated hepatocellular carcinoma (HCC) has been observed in mice treated with rAAV2, which, according to the applicant, is likely a species and age dependent event (Donsante 2007). HCC has also been observed in adult mice with liver injury induced by high fat diet that were infected with rAAV. This is thought to be a species dependent effect as mice appear to have a lower threshold for HCC development than humans (Dawaldi 2021).

There are observations of liver integration of AAV genomes in various mouse models. Relevance to human risk is confounded by inter-study variability, vector construct dependencies, murine specific integration sites and murine specific physiology (higher ploidy than typical liver diploid cells, contribution of disease progression, mouse strain specific spontaneous tumour formation) and for younger animal the fast growth rate, which could favor integration (Zhong 2013; Chandler 2015; Bell 2006).

In dog, in the article of Nguyen et al. 2021, 1741 AAV integration events were found in 5 out of 6 dogs with 44% of those integrations located near genes involved in cell growth. No liver tumors were observed in all treated dogs after 10 years of observation following administration. There was a correlation between DNA copy number and number of integration events detected. Integrations events were distributed across the canine genome. However, clonal expansions were observed with integrations near genes previously associated with growth control. This clonal dominance was observed among cells transduced by the vector, translating to increase in FVIII levels, which is reminiscent of insertional mutagenesis mechanism.

Other studies on haemophilic dogs treated with rAAV did not reveal cases of HCC in dogs treated with FIX gene therapy followed for 8 years (Niemeyer, 2009).

Apart from the animal model, it should be mentioned that technical achievement in high throughput sequencing and library generation have changed the current view of insertion events for rAAV. This needs consideration since the level of integration observed is at least 3 logs higher than what considered before, regardless the model explored.

Levels of integration were observed after BMN 270 administration in non-human primate liver samples evaluated for up to 26 weeks after treatment (BMN 270-20-013). Analysis of integration site frequencies showed no evidence of clonal expansion or preferential integration in or near common cancer genes, but the period is brief and this absence should be re-considered on the long term.

None of the treated NHP develop tumour/clonal dominance in the course of the study (6 months max). Considering the long half-life of hepatocytes (around 300 days in human), it was anticipated that clonal dominance would not be observed in such a study, except if integration led to a significant modification of growth characteristics of hepatocytes. Only long term studies would clarify the point.

Up to 10% of all integration sites occurred within the window of 100 kb of the transcription starting sites of cancer related genes. About 3% (on average) of IS were located within a particular cancer related gene, and most of them fell within introns (on average 5,51%) and not exons (0,69%). The location of integrated rAAV sequences is clear. However, the analysis seems to privilege or only consider a disruptive effect. This is relevant for tumour suppressor genes for which LOF (loss of

function) is the bed for transformation. For proto-oncogenes, intron or vicinity integration might modify expression profiles leading to GOF (gain of function).

Overall, the sum of non-clinical data suggest that:

i) hepatocyte integration potential is a proven risk associated with the use of rAAV vectors in liver therapies, observed in different species, rodent (mice) and non-rodent (dog and primate)

ii) some of the integrations do occur in the vicinity of cancer related genes (up to 10% in a window of 100 kb), some of which (3% on average) are located within a cancer related gene.

However, to date, it is recognised that rAAV vector integration associated with insertional tumorigenesis has only been observed in mouse studies. Clonal dominance but without tumorigenesis was observed in treated haemophilic dogs.

The translatability and clinical relevance of the identified vector integration in DNA of liver cells as well as in other body cells (as observed in parotid gland in one patient), to humans needs a longer period of observation to validate that aspect of the safety profile for rAAV.

Based on these data, the CAT is of the opinion that the risk of tumorigenesis, albeit being potential, must be carefully considered. This has been implemented into physicians and patient's information (as part of the SmPC/PL as well as the guides for HCP and patients) and covered through long term monitoring of patients. Finally the Risk of malignancy in relation to vector integration in DNA of body cells is a potential important risk as part of the safety concerns of the RMP (see Clinical Safety section).

As regards **carcinogenicity**, on the basis of recent non-clinical findings observed with another rAAV5 vector sharing similar features (same capsid serotype, same IV route of administration, same range of doses …), the applicant provided complementary information related to a pharmacology study, in which occurrence of histologically confirmed tumors of the liver (adenomas and hepatocellular carcinoma) was observed in DKO mice, treated with a 2.10^{14} vg/Kg dose, after a 52 weeks period.

In this study, the Genomic analysis by TES and WGS revealed vector integration at known mouse proto-oncogenes: indeed, tumour samples from animals treated with 2E14 vg/kg BMN307 (Group C) contained integration sites in the 5'UTR of Hras, Kras, or Met. The integration sites with the highest relative frequencies were identified in tumour samples from mice C2 (Hras IS, 5.247%) and mice C6-2 (Met IS, 8.179%), but other IS in Hras, Kras, or Met have low relative frequencies (< 0.5%).

According to the applicant, quantitative analyses indicated the pattern of vector integration at these sites was not consistent with a single vector insertion having driven tumour initiation and growth. In these tumors, only a small fraction of cells in each tumour contained a given vector integration event and suggests that multiple rounds of vector integration occurred within a single locus in each tumour.

According to the applicant, data are consistent with a transformation event that is separable from vector integration having led to tumour initiation and cellular growth. It is noted that data on expression of Hras, Kras, or Met are not transmitted/available.

Integrations occur at low frequencies, and following a heterogeneous pattern. The CAT is in agreement that tumors seem to display no clonality as far as vector is concerned, supporting that BMN 307 integration inside oncogenes could not be a tumour initiator event. However, it could be nonetheless a contributing factor in the process of tumorigenesis. In that sense, if vector integrates in already emerged tumors, the absence of anomaly in the control animals is notable.

The absence of historical data on the specific strain PAHenu2/enu2 /Rag2-/- mice is acknowledged.

As regards, treated mice to better interpret the heterogeneity of the tumors and refine the potential

implication of the vector in this, a phylogenetical analysis on tumors found in rAAV treated mice would have been useful but at this stage the information provided by this study would only be complementary.

In addition, a comparative analysis on genome of control mice, versus treated mice at the same age by WGS could inform on the genetic background of the mouse strain, and its propensity to develop tumors.

Roctavian is considered to have an overall negligible environment impact.

2.5.7. Conclusion on the non-clinical aspects

Valoctocogene roxaparvovec can be granted a marketing authorisation from a non-clinical point of view.

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

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

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

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

- **Tabular overview of clinical studies**

Study #	Study Design	Study Objective	Dosing Regimen	Duration (subject participation)	Subjects Enrolled*	Status	Last Patient Completion Date (Actual or Projected)	Report Data Included in MAA
Non-Interventional Studies								
270-901	Observational	To study the seroprevalence of antibodies and neutralizing factors against AAV serotypes in HA patients with residual FVIII activity levels ≤ 2 IU/dL who were previously treated with FVIII concentrates	N/A	Up to 6 months longitudinal sample collection	546	Completed	18 December 2019	Final Report
270-902	Non-Interventional	To prospectively collect bleed, hemophilia medication, and HRQoL information from patients with severe HA on currently available FVIII prophylactic treatment regimens across multiple countries	N/A	Up to 52 weeks	294	Completed	4 December 2019	Final Report
Phase 1/2 Studies								
270-201	Phase 1/2, Open-Label, Dose-Escalation	To assess the safety, tolerability and efficacy of a BMN 270 single IV infusion in patients with severe HA (FVIII ≤ 1 IU/dL)	Single dose at one of four doses: 6E12 vg/kg (N=1) 2E13 vg/kg (N=1) 6E13 vg/kg (N=7) 4E13 vg/kg (N=6)	Approx. 7 years*	15	Ongoing (enrolment completed)	March 2024	Interim full CSR with safety and efficacy from 15 subjects *MA Data Cutoff: 8 April 2020*
270-203	Phase 1/2, Safety, Tolerability, and Efficacy Study	To evaluate the safety, tolerability, and efficacy of BMN 270 in patients with severe HA and pre-existing antibodies against AAV5 vector capsid at various levels of AAV5 antibody titers	Single dose at 6E13 vg/kg	Approx. 5 years	1 enrolled; Approx. 10 planned	Ongoing (enrolling)	June 2027	Interim abbreviated CSR with safety and efficacy from 1 subject *MA Data Cutoff: 14 May 2020*

Study #	Study Design	Study Objective	Dosing Regimen	Duration (subject participation)	Subjects Enrolled*	Status	Last Patient Completion Date (Actual or Projected)	Report Data Included in MAA
270-205	Phase 1/2, Safety, Tolerability, and Efficacy Study in HA Patients with Active or Prior Inhibitors	To assess whether BMN 270 can safely alter the clinical phenotype of HA patients with FVIII activity ≤ 1 IU/dL at the time of detected inhibitors, who have developed FVIII neutralizing antibodies (inhibitors) during HA treatment that are persistent (active) or have resolved (prior)	Single dose at 6E13 vg/kg	Approx. 5 years	Approx. 20-40 planned	Initiated (enrolling)	June 2027	None
Phase 3 Studies								
270-301	Phase 3, Open-Label, Single-Arm	To evaluate the efficacy and safety of BMN 270 at a dose of 6E13 vg/kg in HA patients with baseline FVIII activity levels ≤ 1 IU/dL receiving prophylactic FVIII infusions	Single dose at 6E13 vg/kg	1-year analysis: 52 weeks Long-term follow up: Approx. 5 years	134	Ongoing (enrolment completed)	November 2024	Interim full CSR with efficacy and safety from 134 subjects *MA Data Cutoff: 16 November 2020*
270-302	Phase 3, Open-Label, Single-Arm	To evaluate the efficacy and safety of BMN 270 at a dose of 4E13 vg/kg in HA patients with baseline FVIII activity levels ≤ 1 IU/dL receiving prophylactic FVIII infusions	Single dose at 4E13 vg/kg	Final analysis: 52 weeks Long-term follow up: Approx. 5 years	1	Ongoing (enrolment completed)	May 2023	Interim abbreviated CSR with safety and efficacy from 1 subject *MA Data Cutoff: 20 June 2020*
270-303	Phase 3b, Open Label, Single-Arm	To evaluate the efficacy and safety of BMN 270 with prophylactic corticosteroids in HA patients	Single dose at 6E13 vg/kg	Approx. 5 years	5 enrolled; Approx. 20 planned	Ongoing (enrolling)	Sept 2026	None

* The CSR for 270-201 had a data cutoff in April 2020; subsequent to the data cutoff, the protocol for 270-201 was amended to extend the study duration to approximately 7 years of post-infusion follow-up (Protocol Amendment 9, dated 16 June 2020)

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

BMN-270 is a viral gene therapy product to be administered intravenously. Traditional pharmacokinetic analyses do not apply to products that form in vivo, and therefore no clinical studies have been conducted to investigate the classical aspects of absorption, metabolism or excretion of BMN 270.

2.6.2.2. Pharmacodynamics

BMN 270 is a recombinant, replication-incompetent, AAV5 vector containing a DNA genome. The vector genome includes double stranded inverted terminal repeats (ITRs) at its 5' and 3' ends and single stranded DNA encoding a hybrid human liverspecific promoter (HLP); a B domain deleted (BDD) human FVIII (hFVIII) gene (McIntosh 2013); and a synthetic polyadenylation signal (SpA; Levitt 1989). The genome is contained within an icosahedral capsid composed of the three AAV structural proteins, VP1, VP2, and VP3. The encoded protein consists of a leader sequence; a heavy chain containing the A1 and A2 domains; an SQ domain comprised of 14 amino acids of linker sequence (Sandberg 2001), which is a part of the B domain of wild-type FVIII and replaces the whole sequence of B domain; and a light chain containing the A3, C1, and C2 domains.

- *Quantitative Measurement of FVIII Activity in Human Plasma*

Factor VIII (FVIII) activity was measured by a central laboratory using a validated chromogenic substrate assay (CSA) Coatest® SP and a validated One-Stage clot (OS) assay using a modification of the activated partial thromboplastin time (APTT) method on the Siemens BCS® XP analyzer. CSA was selected as the primary endpoint to evaluate efficacy of BMN 270 during the interim analysis. Following BMN 270 infusion, higher OS than CS FVIII activity was consistently observed in human plasma

samples. The discrepancy between FVIII activity results with OS and CS assays was investigated by the applicant explaining it would be due to the accelerated initial rate of FXa formation for BMN 270 transgene-produced FVIII-SQ compared to native FVIII. The assay used for FVIII activity measures of BMN 270 in clinical studies was CS. The section 4.4 of the proposed SmPC indicates that either OS and CS assay may be used with a conversion factor OSA = 1.5 x CSA; OSA results was on average being approximately 1.636 and 1.43-fold higher than the CSA results in studies 270-201 and -301 respectively.

- *Vector DNA Shedding and Blood Biodistribution (Studies 270-201 and 270-301)*

The BMN 270 vector DNA biodistribution was assessed by qPCR in blood and the BMN 270 vector DNA shedding was assessed by qPCR in semen, saliva, urine and stool. The biodistribution and shedding of potentially infectious vector DNA were further characterised in plasma and semen by evaluating concentrations of encapsidated vector DNA using immunoprecipitation coupled qPCR (iqPCR). The biodistribution of vector DNA in blood was further characterised through qPCR analysis of PBMC, RBC and plasma fractions of whole blood. The contiguity and structural characteristics of vector DNA in whole blood and PBMCs were further evaluated using droplet digital PCR (ddPCR) methods.

In Interim Clinical Pharmacology Report of studies 270-201 and 270-301, individual data of Biodistribution and Shedding Concentrations were presented in different forms: a corresponding numerical copy number concentration values or BLOQ or BLOD or negative. A better explanation on differences between those definitions as well as back calculations and changes in thresholds were provided by the applicant in response to D120 LoQ.

270-201

In **270-201**, administration of BMN 270 resulted in detectable vector DNA in blood and all shedding matrices evaluated, with peak concentrations observed between 1 and 9 days post BMN 270 administration. The greatest concentrations were observed in blood, followed by saliva, semen, stool and urine. Following administration of 6E13 vg/kg BMN 270, all subjects achieved clearance, defined as 3 consecutive samples below the limit of quantification of the analytical method (BLQ), from saliva, semen, stool and urine. At the resubmission data cut-off, the median [min, max] time to first BLQ sample confirmed by 2 additional consecutive BLQ samples for saliva, semen, stool, and urine were 6.97 [3.97, 12.0] weeks, 5.96 [4.84, 22.0] weeks, 28.4 [20.0, 78.0] weeks, and 2.98 [1.97, 3.96] weeks, respectively. Four out of 7 (57.1%) subjects achieved clearance defined by 3 consecutive BLQs in blood with a median [min, max] time to first BLQ confirmed by 2 additional consecutive samples of 160 [113, 169] weeks.

Table 2 Vector DNA Biodistribution and Shedding After 6E13 vg/kg BMN 270 Administration (Study 270-201)

		No. (%) of Detect. Subjects	Time to First Detect. Sample[a] (wk)	Peak Conc.[b] Median (vg/mL)	Time to Peak Conc. (wk)	Time to Last Detect. Sample[c] (wk)	Duration of Detect. Shedding[d] (wk)	Time to first BLQ/ negative sample confirmed by 2 consec. samples[e] (wk)	Number (%) of subjects with 3 consec. BLQ samples	Time to 3 consec. negative samples[f] (wk)	Time to first negative sample confirmed by 2 consec. samples[g] (wk)	Number (%) of subjects with 3 consec. negative samples
Blood	**N**	7 (100%)	7	7	7	0	0	4	4 (57.1%)	0	0	0 (0%)
	Min		0.146	4.84E+09	0.146	-	-	113		-	-	
	Median		0.149	7.05E+09	0.149	-	-	160		-	-	
	Max		0.151	1.51E+10	0.151	-	-	169		-	-	
Saliva	**N**	7 (100%)	7	7	7	7	7	7	7 (100%)	7	7	7 (100%)
	Min		0.147	1.04E+07	0.147	20.0	19.8	3.97		40.0	27.0	
	Median		0.15	6.19E+07	0.151	28.0	27.8	6.97		44.3	36.0	
	Max		1.98	1.51E+08	1.98	42.0	40.0	12.0		52.0	44.0	
Semen	**N**	7 (100%)	7	7	7	7	7	7	7 (100%)	7	7	7 (100%)
	Min		0.147	4.87E+05	0.423	13.0	12.8	4.84		15.0	12.0	
	Median		0.15	2.95E+06	1.29	20.0	19.8	5.96		20.0	14.8	
	Max		1.98	5.02E+07	2.98	68.9	68.7	22.0		83.1	76.5	
Stool	**N**	7 (100%)	7	7	7	6	6	7	7 (100%)	6	6	6 (85.7%)
	Min		0.146	1.28E+05	0.151	73.1	73.0	20.0		87.1	79.1	
	Median		0.149	5.90E+05	0.973	107	107	28.4		115	107	
	Max		0.151	1.79E+06	1.98	151	151	78.0		130	121	
Urine	**N**	7 (100%)	7	7	7	7	7	7	7 (100%)	7	7	7 (100%)
	Min		0.147	4.49E+04	0.423	2.98	2.83	1.97		5.98	3.97	
	Median		0.15	2.70E+05	1.29	9.97	8.00	2.98		7.98	5.96	
	Max		1.98	1.82E+06	2.96	15.0	14.8	3.96		28.0	16.0	

a Defined to time to first positive shedding sample
b Units for stool reported as vg/mg
c Defined as time to last positive shedding sample followed by a negative
d Defined as time between first positive shedding sample and last positive shedding sample confirmed by a negative
e Confirmed by 2 consecutive negative or BLQ samples
f Reported as time of third sample
g Reported as time of first negative sample confirmed by 2 additional consecutive negative samples.

Further characterisation of vector DNA shedding in semen was performed. Vector DNA was not detected in sperm when tested in subjects with detectable (below the limit of quantitation) vector DNA in semen more than 52 weeks after BMN 270 administration, suggesting the vector DNA detected in semen is not associated with sperm. This finding is consistent with findings from two nonclinical studies conducted in mice that demonstrated no occurrence of germline transmission (BMN 270-19-008 and BMN 270-19-033).

Following administration of 6E13 vg/kg BMN 270 in 270-201, encapsidated vector DNA was detectable in plasma and semen from all 7 subjects who participated in the study.

Peak encapsidated vector DNA concentrations in plasma and semen were observed soon after dose administration, with a median time to peak levels across all participants of 0.420 and 0.554 weeks in plasma and semen, respectively. The median peak capsid concentration was 2.81E+08 vg/mL and 7.72 E+05 vg/mL in plasma and semen, respectively. For subjects with 3 consecutive negative samples tested at the time of this report (7/7 for plasma; 5/7 for semen), the median time to the last positive sample was 1.98 and 2.98 weeks in plasma and semen, respectively. The median [min, max] time to the first of 3 consecutive negatives samples was 3.12 [2.97, 3.99] and 4.12 [1.85, 8.97] weeks in plasma and semen, respectively.

Further characterisation of vector DNA biodistribution in blood was performed with qPCR analysis of fractionated whole blood to determine the compartment within blood that contains vector DNA and with drop-phase ddPCR to determine the form of the vector DNA detected. In blood, the majority of vector DNA present beyond 24 weeks is likely within the long-lived fraction of whole blood including PBMCs. The predominate form of vector DNA transitions from truncated to full-length over time. By 52 weeks postdose, the majority of vector DNA remaining in whole blood is full-length and contains a head-to-tail ITR fusion. These results are consistent with the hypothesis that during the first few weeks after AAV-based gene therapy, unstable DNA forms are degraded while stable episomal forms remain.

In **270-301**, the vector DNA biodistribution and shedding profiles characterised at the time of this MA are overall consistent with the profiles characterised with 6E13 vg/kg BMN 270 in 270-201.

Table 3 Vector DNA Biodistribution and Shedding After 6E13 vg/kg BMN 270 Administration - updated

		No. (%) of Detectable Subjects	Time to First Detectable Sample[a] (wk)	Peak Conc.[b] Median (vg/mL)	Time to Peak Conc. (wk)	Time to Last Detectable Sample[c] (wk)	Duration of Detectable Shedding[d] (wk)	Time to first BLQ/ negative sample confirmed by 2 consecutive samples[e] (wk)	Number (%) of subjects with 3 consecutive BLQ/ negative samples	Time to 3 consecutive negative samples[f] (wk)	Time to first negative sample confirmed by 2 consecutive samples[g] (wk)	Number (%) of subjects with 3 consecutive negative samples
Blood	N	134 (100%)	134	134	134	2	2	7	7 (5.22%)	0	0	0 (0%)
	Median		0.143	4.73E+09	0.143	78	77.9	101		-	-	
	Min		0.143	1.55E+08	0.143	75.9	75.7	31.9		-	-	
	Max		1.00	2.01E+11	1.00	80.1	80.0	130		-	-	
Saliva	N	134 (100%)	134	134	134	126	126	133	133[j] (99.2%)	98	98	98[j] (73.1%)
	Median		0.143	6.63E+07	0.143	40.1	40.0	6.43		49.7	40.1	
	Min		0.143	1.26E+06	0.143	6.00	4.86	3.14		16.0	7.86	
	Max		1.14	4.27E+09	2.29	116	115	26.1		132	122	
Semen	N	133[h] (100%)	133	133	133	128	128	132	132[i] (99.2%)	121	121	121 (90.9%)
	Median		0.143	1.75E+06	1.00	20.0	19.4	6.14		36.0	20.6	
	Min		0.143	1.20E+04	0.143	4.00	3.86	0.571		12.0	6.14	
	Max		2.14	1.02E+10	12.1	81.7	81.6	36.1		111	76.1	
Stool	N	134 (100%)	134	134	134	21	21	112	112 (83.6%)	4	4	4 (2.98%)
	Median		0.143	2.65E+05	1.14	88.0	86.7	44.0		114	101	
	Min		0.143	2.09E+02	0.143	63.3	63.1	12.0		88.4	68.4	
	Max		6.29	5.65E+06	6.29	140	140	88.1		155	144	
Urine	N	134 (100%)	134	134	134	134	134	134	134 (100%)	132	132	132 (98.5%)
	Median		0.143	8.91E+04	0.214	8.00	7.86	2.29		20.0	12.1	
	Min		0.143	7.70E+03	0.143	1.14	1.00	0.286		6.00	3.00	
	Max		0.857	3.69E+07	2.29	44.1	44.0	8.14		57.1	48.0	

[a] Defined as time to first positive shedding sample

[b] Units for stool reported as vg/mg

[c] Defined as time to last positive shedding sample followed by a negative

[d] Defined as time between first positive shedding sample and last positive shedding sample confirmed by a negative

[e] Confirmed by 2 consecutive negative or BLQ samples

[f] Reported as time of third sample

[g] Reported as time of first negative sample confirmed by 2 additional consecutive negative samples

[h] [redacted] did not have available semen shedding assessments

[i] [redacted] had not achieved three consecutive BLQ samples through Week 16, the latest sample available at the time of the datacut date for this updated analysis, the concentration in semen at Week 16 was BLQ

[j] Additional data that became available post data snapshot confirmed one additional subject ([redacted]) achieved first negative at Week 52 and first of the 3 consecutive negatives at Week 68

Vector DNA was detected in all matrices tested in all subjects. Peak vector DNA concentrations were observed shortly after dosing across all matrices, with a median [min, max] time to maximum concentration at 0.143 [0.143, 1.00] weeks, 0.143 [0.143, 2.29] weeks, 1.00 [0.143, 12.1] weeks, 1.14 [0.143, 6.29] weeks, and 0.214 [0.143, 2.29] weeks for blood, saliva, semen, stool, and urine, respectively. Peak vector DNA levels were highest in blood, followed by saliva, semen, stool, and urine. Median [min, max] peak concentrations were 4.73E+9 [1.55E+08 2.01E+11] vg/mL, 6.63E+07 [1.26E+06, 4.27E+09] vg/mL, 1.75E+06 [1.20E+04, 1.02E+10] vg/mL, 2.65E+05 [2.09E+02, 5.65E+06] vg/mg, and 8.91E+04 [7.70E+03, 3.69E+07] vg/mL in blood, saliva, semen, stool, and urine, respectively. Following peak vector DNA concentrations, BMN 270 vector genomes declined in the blood, saliva, semen, stool and urine over the duration of follow-up included in this datacut with 7 (5.22%), 133 (99.2%), 132 (99.2%), 112 (83.6%), and 134 (100%) subjects having reached 3 consecutive BLQ or negative measurements in blood, saliva, semen, stool, and urine, respectively. The median [min, max] time to the first BLQ or negative measurement confirmed by 2 additional consecutive measurements was 101 [31.9, 130] weeks, 6.43 [3.14, 26.1] weeks, 6.14 [0.571, 36.1] weeks, 44.0 [12.0, 88.1] weeks, and 2.29 [0.286, 8.14] weeks for blood, saliva, semen, stool, and urine, respectively. It can be noted that the maximum time to achieve vector DNA clearance, defined as 3 consecutive BLQ samples, in saliva, semen and stool is observed beyond the 6 months of contraception provided in the SmPC. The duration of contraception is adapted to the clearance results observed in patients treated by 6E13 vg/kg BMN 270.

Following administration of 6E13 vg/kg BMN 270 in 270-301, encapsidated vector DNA was detectable in plasma from all 134 subjects, and in semen from 131 of the 133 subjects (98.5%) subjects, with evaluable data.

Table 4 Encapsidated Vector DNA Biodistribution in Plasma by iqPCR Following 6E13 vg/kg BMN 270 Administration (Study 270-301) - updated

Dose (vg/kg)		No. (%) of Detectable Subjects	Peak Conc. (vg/mL)	Time to Peak Conc. (weeks)	Time to Last Detectable Sample (weeks)	No. (%) of Subjects that achieved 3 consecutive negatives	Time to first negative sample confirmed by 2 consecutive samples (weeks)
6E13 (n=134)	Min	134 (100%)	BLQ	0.857	0.857	134 (100%)	1.29
	Median		BLQ	1.14	2.21		3.29
	Max		1.13E+07	4.14	10.0		10.1

Table 5 Encapsidated Vector DNA Shedding in Semen by iqPCR Following 6E13 vg/kg BMN 270 Administration (Study 270-301) - updated

Dose (vg/kg)		No. (%) of Detectable Subjects	Peak Conc. (vg/mL)	Time to Peak Conc. (weeks)	Time to Last Detectable Sample (weeks)	No. (%) of Subjects that achieved 3 consecutive negatives	Time to first negative sample confirmed by 2 consecutive samples (weeks)
6E13 (n=133)[a]	Min	131 (98.5%)	BLQ	0.143	0.143	131[b] (98.5%)	0.429
	Median		8.84E+05	0.571	1.86		3.00
	Max		3.79E+08	4.00	8.14		12.1

BLQ, below the limit of quantification.
a One subject did not provide semen shedding assessments;
b Sufficient quantity of semen was not available in two subjects for iqPCR assessment

Peak encapsidated vector DNA concentrations in plasma and semen were observed soon after dose administration, with a median time to peak levels of 1.14 and 0.571 weeks in plasma and semen, respectively. It should be noted that the median time to peak levels corresponds to the earliest assessment evaluated in plasma (Week 1). With 6E13 vg/kg BMN 270, the median peak capsid concentration was below the limit of quantification in plasma and 8.84 E+05 vg/mL in semen. Across

all participants, the median time to the last positive sample was 2.21 and 1.86 weeks in plasma and semen, respectively. 3 consecutive negative samples were observed in plasma for all 134 subjects evaluated, with a median [min, max] time to first of 3 consecutive negatives of 3.29 [1.29, 10.1] weeks. In semen, 3 consecutive negative samples were observed for 131 of the 133 subjects evaluated, with a median [min, max] time to first of 3 consecutive negatives of 3.00 [0.429, 12.1] weeks. In view of these data presented, it could be concluded that potentially "infectious" vector DNA can be detected in plasma and in semen up to approximately 3 months post BMN 270 administration. Given that the iqPCR assay may overestimate the real "infectious" particle amount, the shedding data are acceptable.

In response to D120 LoQ, the applicant clarified that vector DNA per mL units were used for qPCR assay (evaluating vector DNA) which is identical to vg/mL used for iqPCR assay (evaluating encapsidated (potentially infectious) vector DNA). The applicant specified, for each matrix evaluated, the LLOQ for qPCR assay and the LLOQ and the LOD for iqPCR assay. The duration of contraception is based on clearance time of all vector DNA in semen observed after 3 consecutive results assessed by both methods which have a similar threshold of residual DNA (LLOQ <24 000 vg/mL by qPCR and LOD <23 300 vg/mL) by iqPCR). Indeed, the recommended duration of contraception proposed in the SmPC is based on the time to clearance of encapsidated (potentially infectious) vector DNA in semen (12 weeks) by iqPCR assay plus an additional 3 months to account for a complete spermatogenesis cycle washout period and the time to the first of the 3 consecutive BLOQ results of vector DNA by qPCR assay. The 6 months contraceptive measures are acceptable.

Vector DNA transitioned from its initial truncated form into full-length transgenes over time. By 28 weeks through 52 weeks post-BMN 270 administration, the majority of vector DNA remaining in whole blood and PBMCs was full-length. The median fraction of DNA that contained a head-to-tail ITR fusion, indicating formulation of circularised episomal structures, increased over time. By 28 weeks through 52 weeks following BMN 270 administration, a consistent fraction of vector DNA that contained a head-to-tail ITR fusion in whole blood and PBMCs was observed. Taken together these data provide mechanistic evidence on the assembly of a stable and functional transgene from transduced vector DNA.

2.6.3. Discussion on clinical pharmacology

FVIII activity was measured by chromogenic substrate assay (CSA) and one-stage clot assay (OS) that were both appropriately validated assays. The assay used for FVIII activity measures of BMN 270 in clinical studies was CSA. A discrepancy between FVIII activity results with OS and CS assays was observed and investigated by the applicant who explained it was due to the accelerated initial rate of FXa formation for BMN 270 transgene-produced FVIII-SQ compared to native FVIII. The SmPC indicates that either OS and CS assay may be used with a conversion factor OSA = 1.5 x CSA.

After BMN 270 administration, vector DNA was detected in blood, semen, saliva, urine and stool in all subjects. The profiles characterised to date in 270-301 are consistent with the profiles characterised with 6e13 vg/kg BMN 270 in 270-201. For the same dose of BMN 270 (6E13 vg/kg), a high variability of vector DNA concentrations is observed between the patients treated in 270-201 and those treated in 270-301, whatever the matrices evaluated. Results on clearance in blood support the long time to clearance of clinically meaningful quantities of vector DNA. The maximum time to achieve vector DNA clearance, evaluated by qPCR assay, defined as 3 consecutive BLQ samples, in blood, saliva, semen and stool is observed beyond the 6 months of contraception provided in the SmPC. The duration of contraception is adapted to the clearance results observed in patients treated by 6E13 vg/kg BMN 270. Indeed, the recommended duration of contraception proposed in the SmPC (6 months) is based on the time to clearance of encapsidated (potentially infectious) vector DNA in semen (12 weeks) by iqPCR assay plus an additional 3 months to account for a complete spermatogenesis cycle washout period

and the time to the first of the 3 consecutive BLOQ results of vector DNA by qPCR assay, which is endorsed. For 2 patients treated in 270-301, 35400 vg/mL and 24100 vg/mL in semen were observed beyond 6 months of contraception, at 25.9 weeks and at 31.8 weeks after BMN 270 administration respectively. However, shedding of vector DNA in semen characterised in two patients treated in 270-201 with detectable, below the LOQ vector DNA more than 52 weeks after BMN 270 administration, show that analysis of purified sperm samples collected were negative for vector DNA at Week 56 for one patient and Week 52 for the other patient, suggesting the vector DNA detected in semen is not associated with germline cells. Additional data from 149 patients (15 patients in 270-201 and 134 patients in 270-301) confirm that encapsidated ("infectious") vector DNA is no longer present in plasma after 10.1 weeks. Other data generated in 143 patients (12/15 patients in 270-201 and 131/133 patients evaluated in 270-301) dosed with 6E13 vg/kg show that encapsidated ("infectious") vector DNA is no longer present in semen beyond 12.1 weeks. Further characterisation of vector DNA biodistribution in blood was performed with analysis of fractionated whole blood into PBMCs, RBCs, and plasma. From approximately 24 weeks after BMN 270 administration and beyond, a slower rate of decline in small amounts of vector DNA is observed and the majority of transgene DNA present is within the PBMC fraction. Data also indicate evidence of the presence of a stable, full-length transgene from transduced vector DNA in whole blood and the long-term form of DNA in blood is an ITR-fused episomal structure.

Overall all treated subjects were AAV5 Tabs positive within 8 weeks after BMN 270 infusion with no apparent correlation within FVIII activity. Subjects with pre-existing anti-AAV5 antibodies were excluded from the pivotal study while they represented 14.4% of the screened subjects. Three subjects developed detectable anti-AAV5 antibodies during the time between screening and Day 1 prior to infusion and were low-responders at Week 49-52. A total of 12 subjects across all studies had transient positive FVIII TAbs at one or more timepoints and showed a large range of median FVIII activity levels post-BMN 270 at Weeks 49-52 (i.e. from 0 to 207.35 IU/dL), taking into account that none of them had neutralizing antibodies. Study 270-205 is currently in development to investigate safety, tolerability, and efficacy study in HA patients with a history of FVIII inhibitors; as of the MAA submission no subject was enrolled in the study.

The majority of subjects treated by BMN 270 in Studies 270-301, -302 and -303 (76%) were tested positive to AAV5 capsid-specific cellular immune response at one or more timepoints post-administration using the IFN-γ ELISpot assay. Based on the analysis of IFN-γ with ALT and FVIII activity measures, there was no evidence of a correlation between AAV5 capsid-specific cellular immune response and lower FVIII activity. There was no evidence either of a correlation between FVIII specific cellular immune response and lower FVIII activity.

Mean and median FVIII activity levels beyond the 2-year period were extrapolated with a PKPD model, and modelling of the terminal elimination phase suggest an effect up to 5 years. However for further applications and modifications concerning Roctavian, the model will have to be updated using the additional data points that will be available to develop the up model up to 5 years, together with data available from ongoing studies 270-203, -205 and 303.

2.6.4. Conclusions on clinical pharmacology

The product can be approved on pharmacology grounds.

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

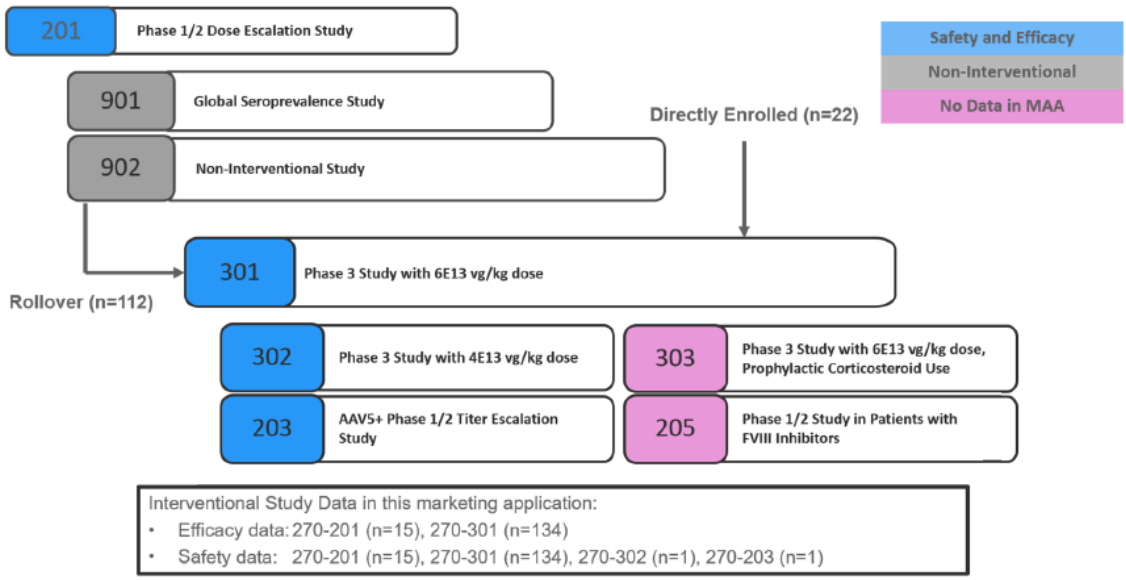
2.6.5. Clinical efficacy

The BMN 270 clinical development programme consists of 6 interventional studies and 2 non-interventional studies. The efficacy data came from the dose-response Study 270-201 and the pivotal Study 270-301 similarly to the previously submitted and subsequently withdrawn MAA (EMEA/H/C/4749). Both studies are ongoing; DCO date was 8 April 2020 for Study 270-201 and 16 November 2020 for Study 270-301.

In the current application, data from Study 270-301 were based on 134 patients (ITT population) who have reached Week 52 while data on only 22 patients (ITT population) reaching Week 26 were presented in the withdrawn application. Additional efficacy data until Week 208 (4 years) were gained from 7 patients of the 15 enrolled in Study 270-201 that were treated at the intended dosage (6E13 vg/kg).

Most of the enrolled subjects in the pivotal study 270-301 were coming from the non-interventional Study 270-902 (N=112, Rollover population) while 22 subjects were directly enrolled.

Figure 1 Summary of BMN 270 Clinical Development Programme



During the procedure updated clinical data from studies 270-201 and 270-301 were submitted:

- 5 years of data for the 6E13 vg/kg cohort and 4 years of data for the 4E13 vg/kg cohort in study 270-201;
- data for all primary and secondary endpoints at 2 years for all subjects in the Phase 3 270-301 pivotal trial, including a subset of subjects who have at least 3 years of data.

2.6.5.1. Dose response study

Study 270-201:

This study was a first-in-human, phase I/II, dose escalation study. The starting dose was selected based on non-clinical studies in mice and monkey to provide pharmacological activity along with a 10-fold safety margin.

Participants were enrolled into one of four cohorts according to dose level. Fifteen subjects enrolled in 270-201 and received BMN 270 in 1 of 4 dose cohorts, in the following order:

- 6E12 vg/kg – 1 subject
- 2E13 vg/kg – 1 subject
- 6E13 vg/kg – 7 subjects
- 4E13 vg/kg – 6 subjects

The first three cohorts were enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation occurred after a single subject in Cohort 1 and Cohort 2 was dosed.

As of the data cutoff for this study (8 April 2020), all 15 subjects remain in post-infusion follow-up. No subjects have discontinued from the study, and no subjects have completed the study. All 15 subjects (100%) have completed the post-infusion Week 156 study visit, and 9 subjects (60%) (all except those in the 4E13 vg/kg cohort) have completed the postinfusion Week 208 study visit. The primary efficacy endpoints: responder status and median FVIII activity at Weeks 13-16 post- BMN 270 infusion (where a responder was defined as a subject with median FVIII activity of ≥ 5 IU/dL at that timepoint).

At Weeks 13-16, 7 of 7 subjects (100%) in the 6E13 vg/kg cohort, and 5 of 6 subjects (83.3%) in the 4E13 vg/kg cohort, met the pre-specified responder definition. Neither subject in the 6E12 vg/kg or 2E13 vg/kg cohort met the responder definition.

As of the data cut of 29 March 2021 for Study 270-201, all subjects (n=7) in the 6E13 vg/kg cohort had completed the Week 260 study visit FVIII assessment, and all subjects (n=6) in the 4E13 vg/kg cohort had completed the Week 208 study visit FVIII assessment.

Between these two cohorts, 8 out of 13 (5 out of 7 subjects in the 6E13 vg/kg cohort and 3 out of 6 subjects in the 4E13 vg/kg cohort up to 260 and 208 weeks post- BMN 270, respectively) subjects had FVIII activity levels > 5 IU/dL at the time of data cut (29 March 2021).

Table 6 Mean and Median FVIII Activity at year end for Cohort 6E13 vg/kg (270-201)

	Year 1 (N=7)	Year 2 (N=7)	Year 3 (N=7)	Year 4* (N=6)	Year 5 (N=7)
Mean (SD) by Chromogenic Assay	64.3 (36.0)	36.4 (26.3)	32.7 (32.8)	24.2 (24.7)	11.6 (12.2)
Median by Chromogenic Assay	60.3	26.2	19.9	16.4	8.2
Range by Chromogenic Assay	12.5, 126.6	3.9, 86.0	4.1, 100.1	2.7, 71.2	0.0, 35.0
Mean (SD) by One-Stage Assay	103.8 (62.4)	59.0 (44.7)	52.3 (54.2)	35.4 (34.2)	18.7 (17.5)
Median by One-Stage Assay	88.6	45.7	29.8	23.4	15.7
Range by One-Stage Assay	20.2, 217.5	6.0, 144.9	5.2, 166.9	5.8, 100.2	3.0, 53.3

*One subject did not have an assessment within the window for the end of Year 4 (Day 1429 to Day 1484) due to a laboratory error. Refer to Section 10.2.

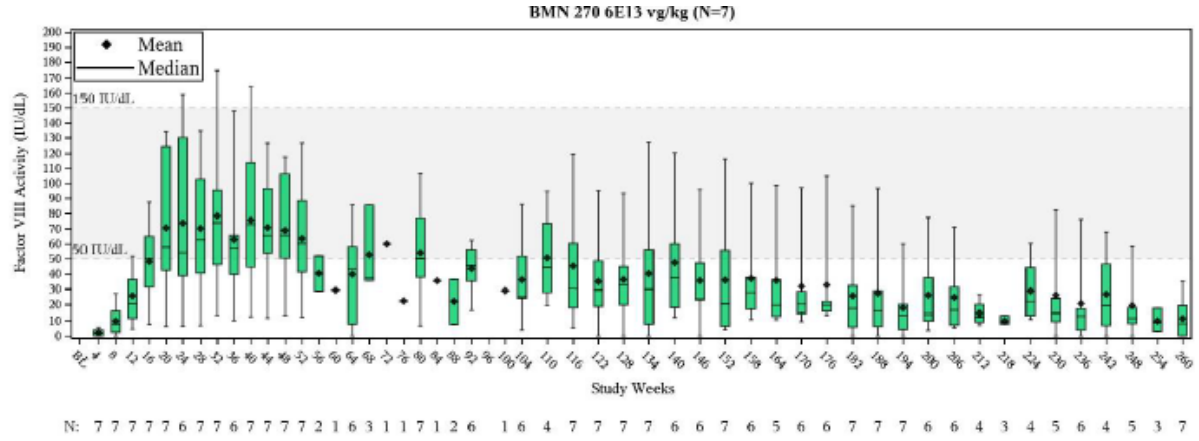
FVIII activity levels below the LLOQ (lower limit of quantitation) are imputed as 0 IU/dL. The LLOQ value for the chromogenic assay is 3 IU/dL, and the LLOQ value for the one-stage assay is 1 IU/dL.

There was a range of median FVIII activity levels (7-122 IU/dL), as measured by chromogenic assay, achieved during Weeks 23-26 among subjects in the 6E13 vg/kg cohort, the range was narrower (4-27 IU/dL) in the 4E13 vg/kg cohort.

The FVIII activity in Cohort 6E13 vg/kg up to Week 260 (5 years) remained sustained, i.e. median FVIII activity level as mesured by CS assay of 8.2 (0.0, 35.0) IU/dL at Week 260. The lower FVIII levels obtained in Cohort 4E13 vg/kg suggested a dose-response to BMN 270. The FVIII activity in

Cohort 4E13 is lower than those in Cohort 6E13 (median FVIII activity level of 4.8 IU/dL at Year 4, i.e. corresponding to moderate HA).

Figure 2 Box Plot for Median Factor VIII Activity Level in 270-201 Using Chromogenic Assay by 4-Week Window (6E13 vg/kg, 270-201)

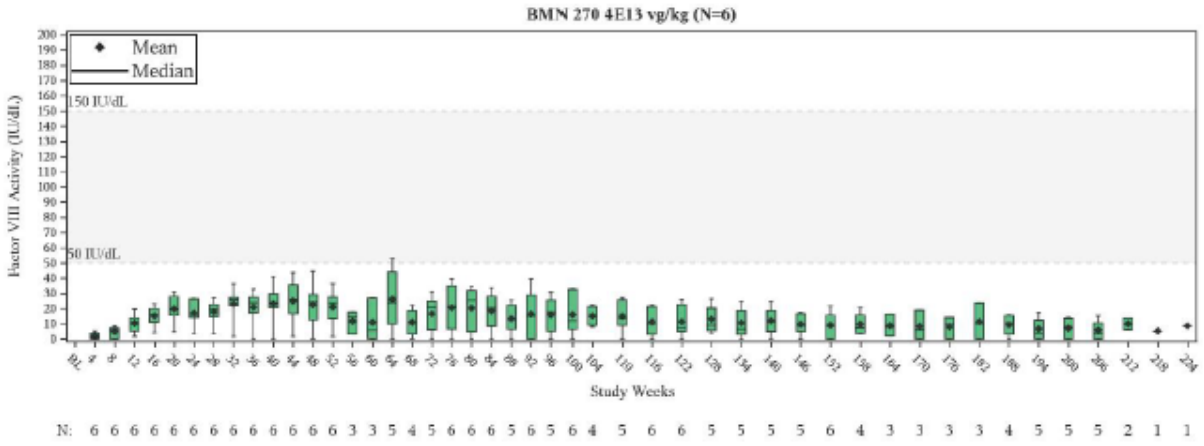


FVIII, Coagulation factor VIII.

Values for FVIII activity were excluded from analysis if obtained within 72 hours (or 3 calendar days if time is not available) since the last infusion of exogenous FVIII replacement therapy. FVIII activity levels below the LLOQ (Lower limit of quantitation – 3 IU/dL for the chromogenic assay) are imputed with 0 IU/dL.

The boxes show the interquartile ranges with the lines in the boxes indicating medians. The ends of the whiskers represent the minimum and maximum values and diamonds indicate the mean.

Figure 3 Box Plot for Median Factor VIII Activity Level in 270-201 Using Chromogenic Assay by 4-Week Window (4E13 vg/kg, 270-201)



FVIII, Coagulation factor VIII.

Values for FVIII activity were excluded from analysis if obtained within 72 hours (or 3 calendar days if time is not available) since the last infusion of exogenous FVIII replacement therapy. FVIII activity levels below the LLOQ – 3 IU/dL for the chromogenic assay) are imputed with 0 IU/dL.

The boxes show the interquartile ranges with the lines in the boxes indicating medians. The ends of the whiskers represent the minimum and maximum values and diamonds indicate the mean.

An increase of subjects with low FVIII activity levels is observed over time consistently with the observed FVIII decline. Three of 6 subjects in the 4E13 vg/kg cohort were low responders at Week

208: and with a median FVIII activity by CSA of 3.0 IU/dL, 0 IU/dL and 0 respectively. Two of the 7 subjects in the 6E13 vg/kg cohort were low-responders, i.e. with a median FVIII activity by CSA of 0 IU/dL at Week 260and with a median FVIII activity of 0 at Week 260.

Table 7 Individual Subject FVIII Activity Levels over Time (Chromogenic Assay) I Activity Levels over Time (Chromogenic Assay)

Subject	Dose (vg/kg)	Week 16 (IU/dL)	Week 26 (IU/dL)	Week 52 (IU/dL)	Week 104 (IU/dL)	Week 156 (IU/dL)	Week 208 (IU/dL)	Week 260 (IU/dL)
[redacted]	6E12	0	0	5.3*	0	7.6* (Week 158)	5.7*	0
[redacted]	2E13	0	0	0	0	0	0	0
[redacted]	4E13	18.0	22.9 (Week 25)	14.3	7.9	3.4	3.0	NA
[redacted]	4E13	8.4	3.0	0	6.1*	0	0	NA
[redacted]	4E13	22.0	25.5	40.1	20.1	22.4	13.7	NA
[redacted]	4E13	24.8	17.6	23.7	10.0	3.9	0	NA
[redacted]	4E13	14.6	27.1	17.7	13.1	10.7	6.2	NA
[redacted]	4E13	15.1	17.1	25.5	22.4	20.5	10.5	NA
[redacted]	6E13	117.0	102.7	49.3	24.6	19.9 (Week 158)	14.5	8.2
[redacted]	6E13	36.4	77.4	62.1	26.2	10.8 (Week 158)	0	0
[redacted]	6E13	68.6	132.5	94.6	38.2	37.6 (Week 158)	18.0	14.1
[redacted]	6E13	61.0	90.4 (Week 28)	88.4	86.0	100.1	62.2	35.0
[redacted]	6E13	31.2	67.2	51.9	24.1	19.6 (Week 162)	11.1	5.0
[redacted]	6E13	65.8	45.9	54.6	51.7	36.2	29.5	19.4
[redacted]	6E13	7.9	6.7	10.6	3.9	0 (Week 158)	3.1 (Week 204)	0

* Subject had resumed prophylactic FVIII therapy therefore this value reflects exogenous FVIII use.

FVIII activity levels below the LLOQ (lower limit of quantitation) are imputed as 0 IU/dL. The LLOQ value for the chromogenic assay is 3 IU/dL, and the LLOQ value for the one-stage assay is 1 IU/dL.

Subjects in the 4E13 cohort have not yet reached Week 260 in the study as of the data cutoff for this report.

Secondary efficacy endpoints:

Annualised Bleeding Rate

As of the data cutoff date of 29 March 2021, clinical data demonstrated a substantial decrease in bleeding events (including zero bleeding events in a majority of 6E13 vg/kg subjects) over 4 and 5 years of observation in the 4E13 vg/kg and 6E13 vg/kg cohorts, respectively, following a single administration of BMN 270 in haemophilia A patients with baseline FVIII activity level ≤ 1 IU/dL. Overall, there was a 96% reduction in mean ABR over 260 weeks post-infusion in the 6E13 vg/kg cohort, and an 86% reduction in mean ABR over 208 weeks post-infusion in the 4E13 vg/kg cohort.

The low-responders and reported higher ABR and exogenous FVIII use from Week 5 to beyond than the other responders; these two subjects reached a FVIII activity level <5 IU/dL earlier than subjects who had a longer hemostatic FVIII activity. The FVIII level increases achieved by the treated-subjects were correlated with the observed reduction of ABR and FVIII utilisation, supporting the clinical relevance of BMN 270.

Table 8 Summary of ABR Requiring Exogenous FVIII Replacement Treatment by Dose Cohort (4E13 and 6E13 vg/kg Cohorts, 270-201)

Cohort	Baseline	Post-Baseline				
		Weeks 1-4		Week 5 and Beyond		
	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Follow-Up (Days)
BMN 270 4E13 vg/kg (N=6)						
Mean (SD)	12.17 (15.35)	1.17 (1.33)	13.32 (15.17)	4.17 (7.36)	1.04 (1.86)	1498.33 (50.03)
Median	8.00	1.00	11.41	1.50	0.36	1498.50
Min, Max	0.0, 41.0	0.0, 3.0	0.0, 34.2	0.0, 19.0	0.0, 4.8	1446.0, 1550.0
BMN 270 6E13 vg/kg (N=7)						
Mean (SD)	17.57 (14.71)	1.57 (1.99)	17.94 (22.69)	3.86 (9.35)	0.77 (1.87)	1830.71 (33.37)
Median	24.00	1.00	11.41	0.00	0.00	1830.00
Min, Max	0, 40	0, 6	0, 68.5	0.0, 25.0	0.0, 5.0	1782.0, 1894.0

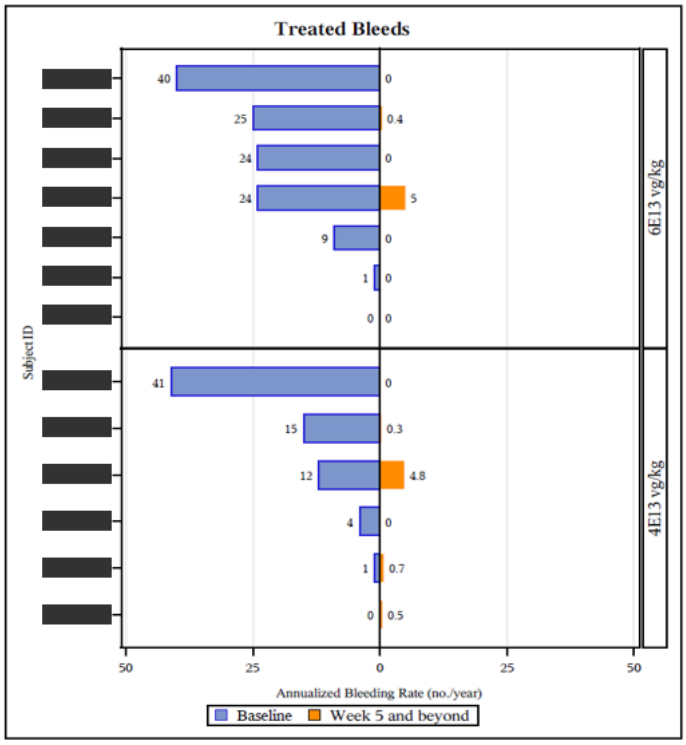
Max, maximum; Min, minimum; SD, standard deviation; Q1: 25% Percentile; Q3: 75% Percentile; CFB: Change from baseline; ABR, annualized bleeding rate.

A bleeding episode is defined as a bleed or symptoms associated with the development of a bleed (or multiple bleeds occurring in the same day) requiring FVIII replacement treatment.

The annualized number of bleeding episodes, or annualized bleeding rate is defined as (Number of bleeding episodes during the calculation period / Total number of days during the calculation period) * 365.25.

Pre-infusion ABR was based on 12 months of historical data collected at Screening visit.

Figure 4 Pre- and Post-BMN 270 Infusion ABR (Treated Bleeds) (270-201)



Exogenous FVIII Usage

As of the most recent data cut (29 March 2021), the median annualised FVIII usage from Week 5 to the data cutoff for the 6E13 vg/kg dose level was 3.19 IU/kg/yr, compared with 5085.90 IU/kg/yr pre-infusion; and the median annualised FVIII usage from Week 5 to the data cutoff for the 4E13 vg/kg dose level was 36.73 IU/kg/yr, compared with 4944.56 IU/kg/yr pre-infusion.

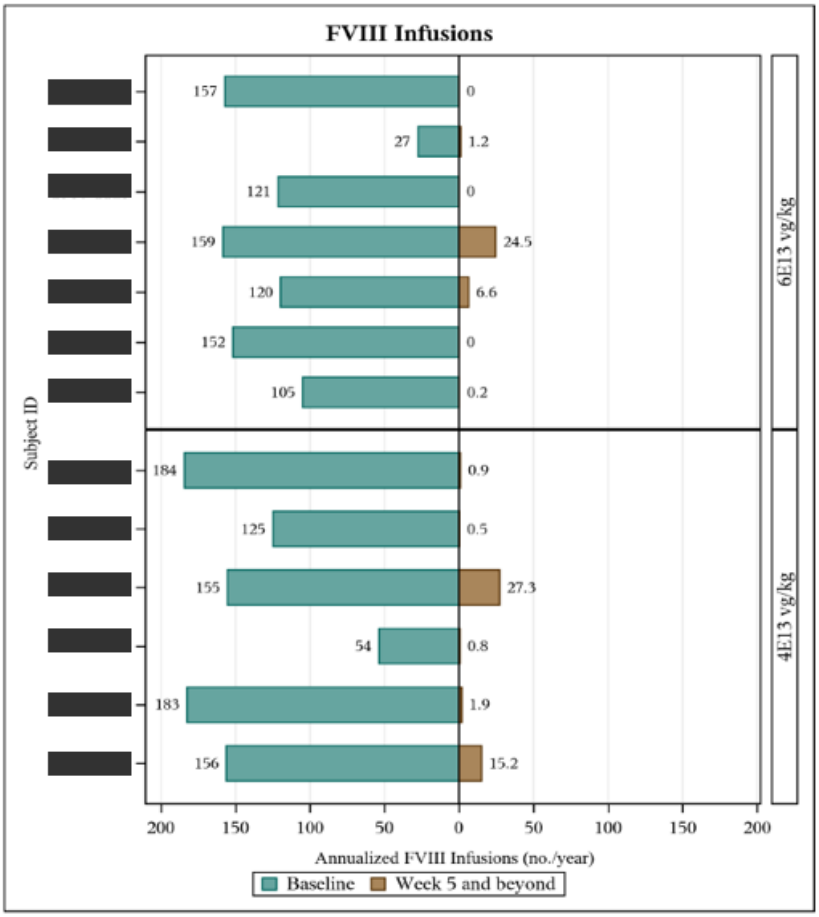
Table 9 Summary Table of Pre-Infusion and Post-Infusion Annualised FVIII Utilisation by Cohort (4E13 and 6E13 vg/kg Cohorts, 270-201)

	Baseline	Post-Baseline				
		Weeks 1-4		Week 5 and Beyond		
Cohort	Annualized FVIII Usage (IU/kg/yr)	FVIII Usage (IU/kg)	Annualized FVIII Usage (IU/kg/yr)	FVIII Usage (IU/kg)	Annualized FVIII Usage (IU/kg/yr)	Follow-Up (Days)
BMN 270 4E13 vg/kg (N=6)						
Mean (SD)	4704.16 (2345.75)	125.48 (108.21)	1432.19 (1235.14)	1055.40 (1681.66)	261.63 (423.81)	1498.33 (2155.50)
Median	4944.56	114.54	1307.35	155.67	36.73	1498.50
Min, Max	1241.4, 7682.7	0, 304.8	0, 3478.8	52.0, 4256.6	13.1, 1075.2	1446.0, 1550.0
BMN 270 6E13 vg/kg (N=7)						
Mean (SD)	4444.48 (1969.50)	85.34 (76.03)	974.10 (867.87)	824.68 (1714.00)	163.66 (342.02)	1830.71 (33.37)
Median	5085.90	62.71	715.76	15.59	3.19	1830.00
Min, Max	573.2, 6438.5	0, 222.8	0, 2542.7	0.0, 4629.3	0.0, 924.0	1817.00, 1838.00

Max, maximum; Min, minimum; SD, standard deviation; Q1: 25% Percentile; Q3: 75% Percentile; CFB: Change from baseline.

Annualized FVIII utilization (IU/kg/yr) = (sum(FVIII replacement treatment (IU/kg)) of the period/sum(follow-up days of the period))*365.2

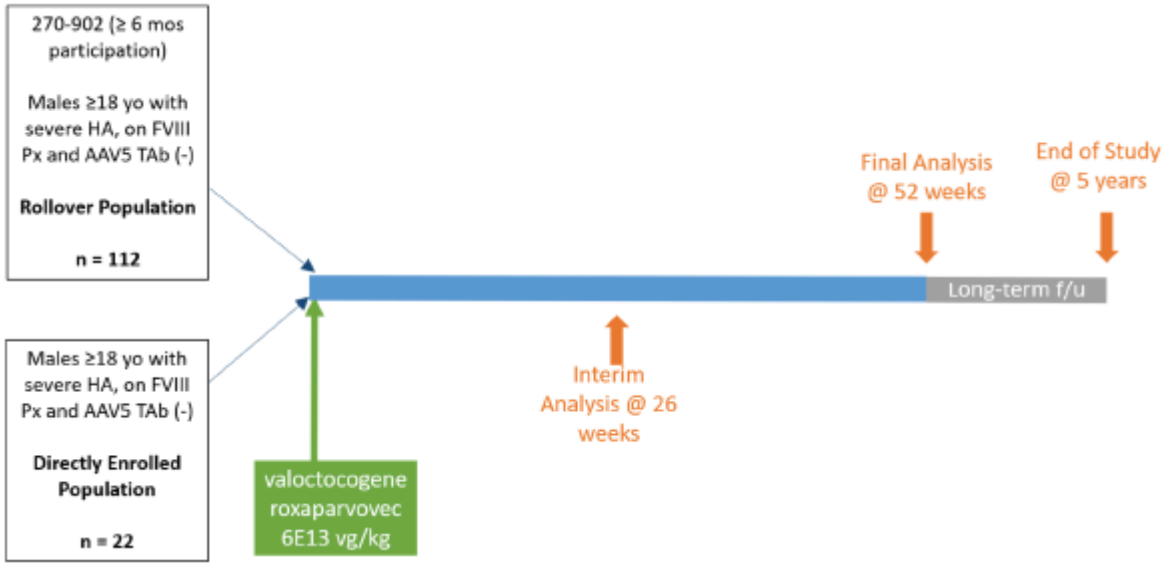
Figure 5 Pre- and Post-BMN 270 Infusion Annualised FVIII Infusions (270-201 4E13 vg/kg and 6E13 vg/kg Cohorts)



2.6.5.2. Main study

Study 270-301: Phase 3, single-arm, open-label study in Haemophilia A patients with residual FVIII levels ⩽ 1 IU/dL treated continuously with prophylactic exogenous FVIII for a minimum of one year prior to enrolment.

Figure 6 270-301 Study Schema



Methods

- **Study Participants**

Individuals eligible to participate in this study must be:

- males ≥ 18 years of age with haemophilia A and residual FVIII levels ≤ 1 IU/dL as evidenced by medical history;
- have been on prophylactic FVIII replacement therapy for at least 12 months prior to study entry with high-quality, well-documented historical data concerning bleeding episodes and FVIII usage over the previous 12 months must have been available. Emicizumab has not been considered in the study. It is a new therapeutic option indicated as prophylactic treatment since 2019 in a similar population to maintain the homogeneity of the subjects' baseline regarding the treatment regimen for their severe haemophilia A. There is large difference in the half-life of emicizumab and FVIII concentrates leading to a larger wash-out period for emicizumab
- treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days;
- have had no previous documented history of a detectable FVIII inhibitor;
- no detectable pre-existing antibodies to the AAV5 capsid;
- no evidence of active infection or any immunosuppressive disorder, including HIV infection. The HIV-positive subjects were initially permitted in the original protocol provided the subject had a CD4 count > 200/mm3 and an undetectable viral load; HIV-positive patients

were later excluded within the Protocol amendment 3, August 2018 after an HIV-positive subject in 270-302 developed markedly elevated liver enzyme. The applicant committed to enrol HIV-infected patients in future studies and precise that work to amend protocols for enrolling studies to enable inclusion of HIV-infected patients is ongoing and should be completed for all protocols by July 2021;

- no significant liver dysfunction/hepatic disease. Indeed AAV are associated with effects of hepatic inflammation;
- no history of arterial or venous thromboembolic events.

- **Treatments**

All subjects received an unique administration of BMN 270, a sterile, clear, colorless-to-pale yellow solution for IV infusion supplied in a 10 mL Crystal Zenith® (CZ) vial. In Study 270-301, subjects were enrolled at a dose of 6E13 vg/kg based on clinical experience in Study 270-201.

The following medications were prohibited starting 30 days before Screening and through the end of the study:

- Any investigational therapy
- Emicizumab
- Fitusiran
- Concizumab
- Efavirenz

The previously prohibited medications non-corticosteroid systemic immunosuppressive agents and lamivudine were removed from the list in the Protocol Amendment 6 dated 3 April 2020. Following dosing with BMN 270, non-steroidal systemic immunosuppressive agents may be used, following a discussion between the Investigator and the Medical Monitor, if corticosteroid use for the treatment of elevated hepatic transaminases has been clinically deemed to be ineffective, not tolerated, and/or contraindicated by the Investigator. Lamivudine was added as a prohibited medication after an HIV-positive subject in a BMN 270 study developed severe ALT elevations while receiving anti-retroviral therapy that included lamivudine as one of its components (and out of concern that lamivudine might be interacting with BMN 270 to exacerbate ALT elevations). However, after discussion with a liver health advisory board, lamivudine is not viewed as a likely medication that would interact with BMN 270.

Subjects were advised to avoid the following, starting 30 days prior to and for at least 52 weeks after BMN 270 infusion, and to minimize their use throughout the remaining duration of the study:

- Alcohol
- Herbal and natural remedies and dietary supplements
- Medications which may be hepatotoxic, including isotretinoin and dextroamphetamine/amphetamine
- Medications which may reduce or increase the plasma concentration of corticosteroids

The following medications should be avoided during oral corticosteroid therapy: Vaccines, NSAIDs.

Subjects were to discontinue their regular FVIII prophylaxis regimen 4 weeks after the day of infusion and switch to an episodic schedule. FVIII replacement therapy could always be taken as needed by the subject for treatment of an acute bleeding episode; in such a case, the subject was asked to record his

treatment and bleeding episodes in his diary. Prophylactic FVIII use was employed as needed on a case-by-case basis and in consultation with the Medical Monitor to prevent bleeding in extenuating circumstances (eg, peri-operative).

Corticosteroid Treatment of Elevated Hepatic Enzymes

Reactive oral corticosteroids (prednisone or converted equivalent) were to be initiated post- BMN 270 if ALT > ULN or ALT 1.5x BL. Reactive oral corticosteroids were primarily initiated if ALT ≥ 1.5xULN, and later amended to initiate reactive corticosteroids if ALT ≥ 1.5x ULN or ALT > ULN and >2x the subject's baseline ALT. A more conservative approach to manage the risk of elevation ALT was adopted further to the interim analysis within the Protocol Amendment 6, which lowered the threshold. Corticosteroids could be delayed if elevations in ALT were clearly not related to BMN 270 (eg, elevated ALT with concurrent increase in creatinine phosphokinase [CPK] due to intensive exercise). The prescribed regimen for reactive oral corticosteroids is detailed in the table below were to be made as follows:

Table 10 Adjustments to Corticosteroid Regimen

Corticosteroids should be tapered on an individual subject basis with the following guiding principles:	Corticosteroids may be tapered if: • ALT ≤ 1.5x baseline value; and • FVIII activity levels > 90% of the pre-decline FVIII activity levels; and • There is no concern for adrenal insufficiency post-withdrawal
Increasing Corticosteroid Dose	If ALT level is increasing or FVIII level is decreasing while on oral corticosteroids, any increases in oral corticosteroid dosing should be made only upon consultation with the Medical Monitor

For any scenarios that are not accounted for in the above table, a discussion should take place between the Investigator and Medical Monitor regarding corticosteroid dose adjustments.

After discontinuation of oral corticosteroids, labs for ALT and FVIII levels were measured once a week for 4 weeks to ensure stability in values.

- **Objectives**

The primary efficacy objective of the study is to:

- Assess the efficacy of BMN 270 defined as FVIII activity, as measured by chromogenic assay, during Weeks 49-52 following intravenous infusion of BMN 270

The secondary efficacy objectives of the study are to:

- Assess the impact of BMN 270 on usage of exogenous FVIII replacement therapy from Week 5 to the last visit as of the data cutoff
- Assess the impact of BMN 270 on the number of bleeding episodes requiring exogenous FVIII replacement therapy from Week 5 to the last visit as of the data cutoff

The tertiary efficacy objective of the study is to:

- Assess the impact of BMN 270 on patient-reported outcome (PRO) measures at Week 52 of the study compared to baseline

The safety objectives of the study are to:

- Evaluate the safety of BMN 270 during the first 52 weeks following intravenous infusion
- Assess the long-term safety of BMN 270

The exploratory objectives of the liver biopsy substudy are:

- To examine the histopathology of the liver following BMN 270 therapy, including assessing for possible safety findings (eg, fibrosis, fatty liver disease, lymphocytic invasion)
- To quantify FVIII DNA, RNA, and protein expression within hepatocytes
- To determine which forms of recombinant adeno-associated virus (rAAV) vector DNA are present at the time of biopsy.
- To determine the transduction pattern of BMN 270 in humans (ie, peri-portal hepatocytes, central vein hepatocytes)

The optional liver biopsy was added in Protocol amendment 6, 3 April 2020 to gain knowledge in the BMN 270 transduction in hepatocytes and establish a correlation with the treatment response.

- **Outcomes/endpoints**

- *Primary endpoint:*

Change of hFVIII activity by chromogenic assay during Weeks 49-52 post-BMN 270 infusion from baseline. If a subject used FVIII within 72 hours of a measurement day, all efforts were made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

As previously assessed, FVIII activity has been recognised as a marker of efficacy with established correlation with clinical endpoints. The approach of defining the primary endpoint on the surrogate, FVIII activity level, has been endorsed by the CHMP in scientific advice for Roctavian.

Subjects who do not respond to BMN 270 treatment (ie treatment failure, manifesting as either failure to achieve FVIII activity $\geq$ 5 IU/dL by Week 52 or inability to maintain independence from prophylactic FVIII replacement therapy due to joint bleeding episodes, in the judgment of the Investigator) may, at the Investigator's discretion and after discussion with the Medical Monitor or Sponsor-designated Data Monitor, follow an abbreviated visit schedule during Years 2-5.

- *Secondary endpoints:*
    - Change of the annualised utilisation (IU/kg) of exogenous FVIII replacement therapy during Week 5 to the last visit as of the data cutoff post-BMN 270 infusion from the baseline utilisation of exogenous FVIII replacement therapy.
    - Change in the annualised number of bleeding episodes requiring exogenous FVIII replacement treatment during Week 5 to the last visit as of the data cutoff post- BMN 270 infusion from the baseline ABR.

The secondary endpoints (annualised utilisation of exogenous FVIII and ABR) are considered clinically relevant to evaluate the effect of BMN 270 on patients with severe haemophilia A.

- *Tertiary endpoint:*
    - Patient-reported Outcome (PRO) measures: Haemo-QoL-A, EQ-5D-5L, EQ visual analogue scale (EQ-VAS), Haemophilia Activities List (HAL), Work Productivity and Activity Impairment plus Classroom Impairment Questions: Hemophilia Specific (WPAI+CIQ:HS) and Patient Reported Outcomes, Burdens, and Experiences (PROBE)

- **Sample size**

Sample size determination

The sample size was updated several times with different protocol amendments. In the original protocol (14th August 2017), approximately 40 subjects were to be dosed in the study. With protocol

amendment 1 (2nd October 2017), the sample size was increased to 70 subjects. From protocol amendment 2 (28th June 2018), 130 subjects were to be dosed in the study.

The latest sample size determination (approximately 130 subjects) was based on clinical and statistical considerations in order to provide sufficient data to assess both safety and efficacy of BMN 270.

For the determination of the sample size, three efficacy endpoints were considered:

- #1: Change of the hFVIII activity, as measured by chromogenic assay, during Weeks 49-52 post-BMN 270 infusion from baseline.
- #2: Change in the annualised utilisation (IU/kg) of exogenous FVIII replacement therapy during Weeks 5-52 post-BMN 270 infusion from baseline
- #3: Change in the annualised number of bleeding episodes requiring exogenous FVIII replacement treatment during Weeks 5-52 post-BMN 270 infusion from baseline

For the primary endpoint, a sample size of 130 provides at least 95% power to demonstrate that the change in hFVIII activity during Weeks 49-52 from baseline is greater than 0, assuming an effect size of 0.6, using a one-sample t-test with a 2-sided significance level of 0.05. The effect size of 0.6 was assumed based on Study 270-201 data.

For the secondary endpoints, the analysis was performed utilizing exogenous FVIII use and bleeding episode data from the approximately 110 HIV-negative subjects whose baseline data was prospectively collected for approximately 6 months in the non-interventional study 270-902 prior to their enrolment in 270-301. An analytic sample size of 110 provides at least 95% power to demonstrate that the change in the annualised utilisation (IU/kg) of exogenous FVIII replacement therapy during Week 5 to Week 52 post-BMN 270 infusion compared to baseline is less than 0, assuming an effect size of 0.6 using one-sample t-test with a 2-sided significance level of 0.05.

For the analytic sample size calculation of the second secondary endpoint, ABR, it was assumed that the pre- and post-BMN 270 infusion population mean ABRs are 3.5 and 1, respectively, and the distribution of ABRs was negative binomial distribution with a dispersion parameter of 2.2. Given the underlying negative binomial distributions, the standard deviations of the pre- and post-BMN 270 infusion ABRs were calculated as 7.8 and 1.8, respectively. The mean (SD) of the change from the pre- to post-BMN 270 infusion ABRs were calculated as -2.5 (8), assuming the correlation between pre- and post-BMN 270 infusion ABRs is zero. Under this assumption, an analytic sample size of 110 also has at least 95% power to demonstrate that the change in the annualised number of bleeding episodes requiring exogenous FVIII replacement treatment (ABR) during Week 5 to Week 52 of the study post-BMN 270 infusion compared to baseline is less than 3.5 (non-inferiority margin), using a one-sample t-test with a 2-sided significance level of 0.05. Under the same assumptions, a sample size of 110 has approximately 90% power to demonstrate that the change is less than 0 (ie, superiority of BMN 270 against FVIII prophylaxis).

Overall, the planned sample size has greater than 80% power for testing the primary and secondary efficacy endpoints hierarchically at the final analysis with a 2-sided significance level of 0.05.

Regarding the second secondary endpoint (ie, the non-inferiority test for change in ABR), in the pivotal studies of recently approved FVIII replacement products, the estimated ABRs are consistent across different studies and products. The mean ABRs of prophylactic treatment groups range from approximately 3 to 6, and the mean ABRs of episodic treatment groups range from approximately 30 to 60. The non-inferiority margin of 3.5 was chosen to preserve 90% of the efficacy of prophylactic over episodic treatment.

- **Randomisation and Blinding (masking)**

This is a non-randomised open-label single arm study.

The applicant implemented some access restrictions to primary and secondary efficacy variables. It is appreciated that these measures are intended to minimize bias, however it cannot be excluded that changes to the study design or planned analyses are influenced by partial information available at the trial sites. Decisions affecting key aspects of the study design cannot be considered independent from the data collected while the trial was ongoing.

- **Statistical methods**

The statistical methods described below correspond to the latest statistical analysis plan (v3, 17th December 2020). It should be noted, that several important changes were made to the statistical methods compared to the original study protocol, this is further discussed below.

Analysis populations

The following populations have been defined:

- **Intent-to-Treat (ITT)** Population (n=134) – all subjects dosed in 270-301
- **Modified Intent-to-Treat (mITT)** Population (n=132) – all HIV-negative subjects dosed in 270-301
- **Rollover** Population (n=112) – all subjects dosed in 270-301 who previously participated in 270-902 (all subjects were HIV-negative)
- **Directly Enrolled** Population (n=22) – all subjects dosed in 270-301 who did not previously participate in 270-902
- **Directly Enrolled HIV-Negative** Population (n=20) – all HIV-negative subjects dosed in 270-301 who did not previously participate in 270-902

Adjustment for multiplicity and interim considerations

Two interim analyses were planned after approximately 16 and 20 treated HIV-negative subjects had completed the Week 26 visit (or had discontinued study prior to Week 26), respectively. The second interim analysis was to occur only if the result from the first interim analysis was not positive (i.e. if the pre-specified statistical significance was not achieved).

The primary efficacy endpoint for the interim analysis was change of the hFVIII activity from baseline, as measured by chromogenic substrate assay, during Week 23-26 post-BMN 270 infusion. The hypothesis testing was to be carried out at the significance level of 0.005, respectively, at the first and second interim analyses. The secondary and tertiary endpoints were to be summarised descriptively at the interim (Week 26) analyses.

To control the family-wise Type I error rate for the interim and the final analyses, the fallback procedure was implemented as follows. For primary analysis of the primary efficacy endpoint:

- If the first interim p-value is ≤0.005, the first interim result is declared significant and the final analysis is carried out at the 0.05 level.
- If the first interim p-value is >0.005, the first interim result is not declared significant and the second analysis is carried out at 0.005 level. If the second interim p-value is <=0.005, the second interim result is declared significant, and the final analysis is carried out at the 0.045 level.

- If the second interim p-value is >0.005, the interim results are not declared significant, and the final analysis is carried out at the 0.04 level.

Regardless of the interim analyses results, the study was to continue to completion with the final analysis performed at Week 52.

For controlling the probability of a type I error for the primary and secondary efficacy endpoints at the final analysis, a hierarchical (sequential) multiple comparison procedure was used, after applying the fallback procedure. Specifically:

- If the first interim result is significant, then at the final analysis the primary and secondary endpoints will be tested hierarchically using alpha = 0.05.
- If the first interim result is not significant but the second interim analysis is significant, then at the final analysis the primary and secondary endpoints will be tested hierarchically using alpha = 0.045.
- If neither of the two interim analysis results is significant, then at the final analysis the primary and secondary endpoints will be tested hierarchically using alpha = 0.04.

The first interim analysis was performed in May 2019. Since the result from the first interim analysis was positive, the second interim analysis did not occur. According to the above procedure, the primary and secondary efficacy endpoints were tested sequentially using alpha = 0.05.

Primary analysis

The primary endpoint, change in the hFVIII activity during Weeks 49-52 post-BMN 270 infusion from baseline, was tested using a one-sample t-test on the mITT population.

The hypotheses were: H0 (null): Change = 0 versus H1 (alternative): Change ≠ 0.

Each subject's hFVIII activity level during Weeks 49-52 was defined as the median of the values obtained during this 4-week window.

The baseline value was imputed as 1 IU/dL. The rationale for the imputation provided by the applicant is that there was no washout of severe haemophilia A subjects' usual FVIII prophylaxis prior to BMN 270 infusion. Post-BMN 270 infusion values for FVIII activity were excluded from analysis if obtained within 72 hours (or 3 calendar days if time is not available) since the last infusion of exogenous FVIII replacement therapy.

If any subject had no assessment available during Weeks 49-52, the last observation carried forward (LOCF) approach was used to impute the missing value. Specifically, the median value in the subject's last 4-week window prior to Weeks 49-52 containing a valid observation was used to impute the missing value. If no such assessment was available, the baseline value of 1 IU/dL was to be used for imputation, i.e., the change from baseline was to be imputed as 0. FVIII activity levels below the LLOQ were imputed with 0 IU/dL.

The LOCF algorithm for the primary endpoint was not specified in the original protocol, and it is not considered to be a conservative approach to missing data, especially when efficacy is anticipated to deteriorate over time. It was confirmed by the applicant that only 2 patients had an imputed value for the primary analysis. The applicant also performed a supplementary analysis where missing post-treatment values are imputed with 0 instead, and the results were consistent.

The descriptive statistics of the primary endpoint indicate that the distribution is positively skewed. Therefore, the one-sample t-test, which assumes data normality, may not be the most appropriate statistic. The applicant was requested to provide sensitivity analyses using a non-parametric test (e.g.

Wilcoxon), and based on log-transformed levels instead. These additional analyses were made available and confirmed the consistency with the primary analysis results.

Secondary analyses

The first secondary efficacy endpoint was the change in the annualised utilisation (IU/kg) of exogenous FVIII replacement therapy during Week 5 - Last Visit by data cut-off post-BMN 270 infusion from baseline

The annualised utilisation (IU/kg) of exogenous FVIII replacement therapy was defined as:

$$\frac{\text{Sum of FVIII use (IU/kg) during calculation period}}{\text{Total number of days during the calculation period}} \times 365.25$$

If the value is missing, e.g., when a subject drops out before Week 5, the value for annualised FVIII utilisation is imputed using the subject's baseline value, i.e., the change is imputed as 0.

The second secondary efficacy endpoint was the change in the annualised number of bleeding episodes requiring exogenous FVIII replacement treatment during Week 5 – Last Visit by data cut-off post-BMN 270 infusion from baseline

The annualised number of bleeding episodes, or annualised bleeding rate (ABR) was defined as:

$$\frac{\text{Number of bleeding episodes during the calculation period}}{\text{Total number of days during the calculation period}} \times 365.25$$

If the value is missing, e.g., when a subject drops out before Week 5, the change in ABR is imputed as the median value of the changes of all subjects' observed cases.

The confirmatory analyses for the secondary endpoints were performed on the Rollover Population. For the second secondary efficacy endpoint, only treated bleeds were considered. Bleeds due to surgery/procedure were not included in this secondary efficacy endpoint.

If the hypothesis testing for the primary endpoint was statistically significant, the secondary endpoints were tested sequentially as described above.

The hypotheses for change in the annualised FVIII utilisation were:

H0 (null): Change = 0 versus H1 (alternative): Change ≠ 0.

The test of annualised FVIII utilisation used a one-sample t-test with sample variance at a two-sided significance level α determined by the fallback procedure.

The first test for change in the annualised number of bleeding episodes requiring exogenous FVIII treatment was a non-inferiority test with a margin of 3.5. The non-inferiority test hypotheses were:

H0 (null): Change ≥ 3.5 versus H1 (alternative): Change < 3.5.

The test of annualised number of bleeding episodes used a one-sample t-test with sample variance at a two-sided significance level α determined by the fallback procedure. A two-sided (1-α)*100% confidence interval of the mean change was provided. If the upper bound of the confidence interval (CI) was less than 3.5, the null hypothesis was rejected.

Subsequently, non-inferiority with smaller margins and superiority were to be assessed using the same CI.

Changes to planned statistical methods

Several important changes were made to the study design and statistical methods of the pivotal trial compared to the original protocol as part of several protocol amendments and SAP versions. The first

SAP version was finalised on 3rd December 2018 (approx. 1 year after study initiation) and subsequent updates were made, the latest version being signed 1 month after the data cut-off for the study report. As described above under "Changes to planned statistical methods", the various changes have impacts on the sample size (increased from 40 to 70 then to 130), the frequency and methods for IAs, the multiplicity adjustment procedure, analysis populations, and primary / secondary analysis definitions. Despite the data access restrictions described by the applicant, data-driven decisions cannot be excluded in the context of an open-label study.

Due to the content and timing of these deviations from pre-specified analyses, it is not possible to consider the study type I error as being formally controlled across primary and secondary analyses. This issue cannot be addressed retrospectively, and as a consequence, the applicant is requested to remove claims of statistical significance / superiority from the SmPC

In this situation, the clinical relevance as well as the robustness of the primary and secondary endpoint analyses will be key to the benefit assessment. More specifically, it is expected that supplementary analyses based on original protocol definitions remain consistent with the analyses based on the final SAP.

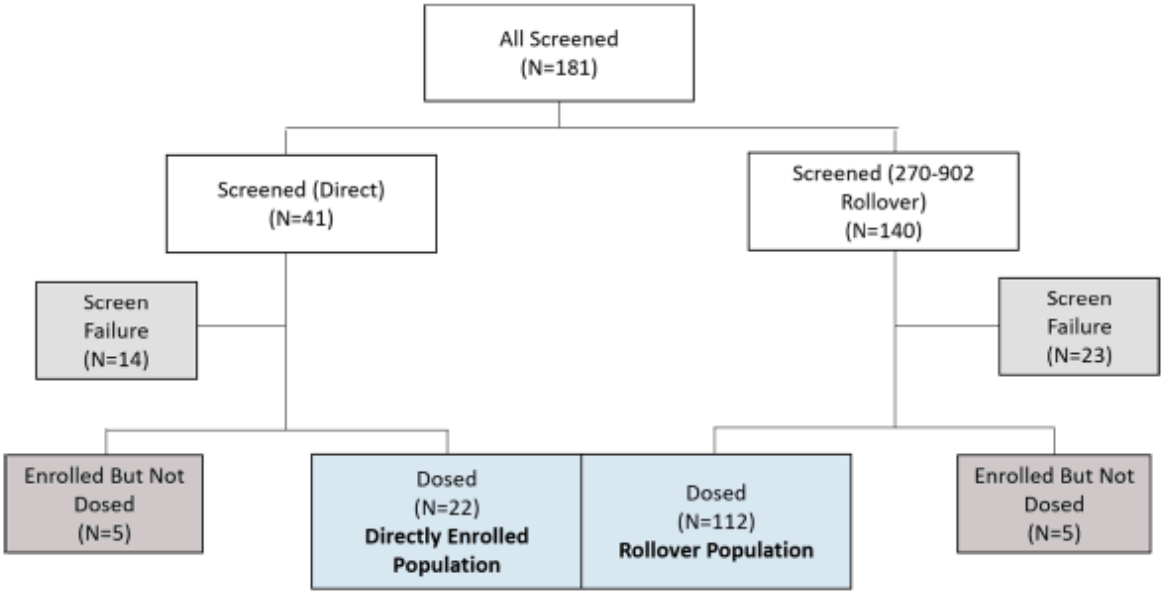
It is unclear which data counted toward baseline bleeding rates and FVIII utilisation. Study 270-902 reports various corresponding rates depending on different definitions of the analysis set and study period. The period from Day 1 in study 270-902 to infusion date in study 270-301 appears most intuitive.

For the primary endpoint, the baseline value was imputed as 1 IU/dL for all subjects. This imputation was not specified in the original protocol and does not reflect the initial intent of a true change from baseline analysis. Technically, the primary analysis ignores baseline and the test is for the post-treatment Week 49-52 hFVIII activity value from which 1 IU/dL is subtracted. It is understood that a trough baseline (>72 hours after last dose of replacement FVIII therapy) may not be measured for all subjects as there is no washout, however it could be used when available. Therefore, the applicant was requested to provide a change from baseline analysis, using actual baseline hFVIII activity values whenever available, as planned in the original protocol. The corresponding results were provided and showed a similar level of mean change from baseline in subjects with non-missing trough baseline values. In subjects with missing trough baseline values, larger FVIII measurements are observed at baseline, with mean (SD) of 12.19 (22.32) IU/dL. As expected, this resulted in lower changes from baseline at week 49-52 compared to the primary analysis results with mean (SD) of 30.70 (49.61) IU/dL. It is noted that the corresponding 95% CI (22.16, 39.25) still excluded 0. It is however acknowledged that, when trough values are not available, these values may not represent the patient's own levels of endogenous FVIII production.

Results

- **Participant flow**

Figure 7 270-301 Participant Flow



One-hundred eighty-one (181) potential subjects were screened for participation in 270-301. The most common reason for screen failure was detectable pre-existing AAV5 antibodies, i.e. 26/181 (14.4%) patients.

Of the 134 dosed subjects, 130 (97.0%) had completed 52 weeks of follow-up on study as of the data cutoff. No subject discontinued prior to Week 52 and all subjects completed the Week 52 visit in whole or in part.

Table 11 Subject Disposition

Category	Directly Enrolled (N=22)	Rollover (N=112)	mITT (N=132)	ITT (N=134)
Subjects treated, n (%)	22 (100)	112 (100)	132 (100)	134 (100)
Continuing in study	21 (95.5)	112 (100)	131 (99.2)	133 (99.3)
Completed Week 52	22 (100)	108 (96.4)	128 (97.0)	130 (97.0)
Discontinued from study	1 (4.5)	0	1 (0.8)	1 (0.7)
Lost to follow-up	1 (4.5)	0	1 (0.8)	1 (0.7)
Discontinued from study before Week 52	0	0	0	0

Disposition as of 16 November 2020

- **Recruitment**

Study start date: 19 December 2017

CSR Data cutoff date: 16 November 2020

There was an additional 19-months follow-up compared to the interim CSR submitted in the withdrawn application.

2-year data cutoff date: 15 November 2021

- **Conduct of the study**

- **Baseline data**

Overall the demographics characteristics were similar between the four populations (directly enrolled, rollover, mITT and ITT). All subjects were male; 48.5% of subjects overall (48.2% of the Rollover Population and 50.0% of the Directly Enrolled Population) were between the ages of 18 and 30. The mean (SD) age at enrollment for the full study population was 31.7 (10.3) years; the oldest subject was 70 years old.

Table 12 Demographics

Characteristic	Directly Enrolled (N=22)	Rollover (N=112)	mITT (N=132)	ITT (N=134)
Age at enrollment, years				
n	22	112	132	134
Mean (SD)	30.9 (8.7)	31.8 (10.6)	31.4 (10.1)	31.7 (10.3)
Median	29.5	30.0	30.0	30.0
Min, Max	18, 52	19, 70	18, 70	18, 70
Age at enrollment, n (%)				
18 to < 30 years	11 (50.0)	54 (48.2)	65 (49.2)	65 (48.5)
30 to < 50 years	10 (45.5)	45 (41.1)	55 (41.7)	56 (41.8)
≥ 50 years	1 (4.5)	12 (10.7)	12 (9.1)	13 (9.7)
Sex, n (%)				
Male	22 (100)	112 (100)	132 (100)	134 (100)
Female	0	0	0	0
Race, n (%)				
Asian	2 (9.1)	17 (15.2)	19 (14.4)	19 (14.2)
Black or African-American	1 (4.5)	14 (12.5)	15 (11.4)	15 (11.2)
Native Hawaiian or other Pacific Islander	0	1 (0.9)	1 (0.8)	1 (0.7)
White	18 (81.8)	78 (69.6)	94 (71.2)	96 (71.6)
Not provided due to patient privacy	1 (4.5)	2 (1.8)	3 (2.3)	3 (2.2)

All subjects enrolled in 270-301 were receiving prophylactic FVIII at the time of study entry. Subjects did not undergo a formal washout of FVIII concentrate prior to baseline FVIII assessment; as such, efficacy comparisons of FVIII against baseline use an imputed baseline FVIII activity value of 1.0 IU/dL.

Baseline FVIII usage and ABR data were analyzed using two different historical periods and methods for the Rollover Population subjects:

- A 6-month baseline analysis period of data collection in 270-902 (starting at Day 1) up to the BMN 270 infusion in 270-301; and
- A 12-month baseline analysis period that also includes the 6 months of high-quality historical data prior to the Day 1 visit in 270-902. Analyses performed as part of the 270-902 study demonstrated consistency between the data collected on-study and the data provided from historical records, thus supporting the validity of treating the two periods together as a 12-month baseline period for use in 270-301.

Directly enrolled subjects provided 12 months of high-quality, complete historical data on FVIII infusions and bleeds.

All subjects in the Rollover Population had at least 12 months of historical data available (mean [SD] 14.38 [2.16] months, range 12.0-21.6 months). For the Directly Enrolled Population (n=22), the mean (SD) duration of baseline data collected was 13.06 (0.35) months (range 12.4-13.7 months).

However, some differences of baseline values are observed between the Directly enrolled population and the Rollover population. Indeed mean (SD) baseline annualised FVIII usage were 4889.11 (1477.54) IU/kg/year and 3961.17 (1751.47) IU/kg/year respectively and mean (SD) baseline ABR (treated bleeds) of 8.41 (19.90) bleeds/year and 4.83 (6.47) bleeds/year respectively, suggesting a

better controlled disease in the Rollover population compared to the directly enrolled. Nevertheless it should be emphasised that the baseline data were differently gathered between these 2 populations since Rollover baselines came from the non-interventional study 270-902 while Directly enrolled baselines were based on historical data that might be less reliable than a dedicated study. A large range of baseline ABR was observed in both populations, i.e. 0-91.5 in Directly enrolled and 0-33.1 in Rollover population. There was a wider range of baseline annualised FVIII usage (IU/kg/year) in Rollover population compared to Directly enrolled, i.e. 1296.4-11251.1 and 2550.9-2885.0 respectively.

Table 13 Baseline Characteristics

Characteristic	Directly Enrolled (N=22)	Rollover (N=112)	mITT (N=132)	ITT (N=134)
Type of FVIII treatment for hemophilia A, n (%)[a]				
Episodic	0	0	0	0
Prophylaxis	22 (100)	112 (100)	132 (100)	134 (100)
Baseline annualized FVIII usage, IU/kg/year[b]				
Mean (SD)	4889.11 (1477.54)	3961.17 (1751.47)	4111.30 (1747.82)	4113.52 (1738.96)
Median	4785.87	3754.42	3860.30	3860.30
Min, Max	2550.9, 2885.0	1296.4, 11251.1	1296.4, 11251.1	1296.4, 11251.1
Baseline annualized number of FVIII infusions, infusions/year[b]				
Mean (SD)	146.08 (78.91)	135.87 (51.99)	138.12 (57.22)	137.54 (57.04)
Median	119.26	128.56	125.09	121.12
Min, Max	49.3, 358.7	39.5, 363.8	39.5, 363.8	39.5, 363.8
Baseline ABR (treated bleeds), bleeds/year[b]				
Mean (SD)	8.41 (19.90)	4.83 (6.47)	5.43 (10.04)	5.42 (9.96)
Median	0.93	2.80	2.04	2.30
Min, Max	0.0, 91.5	0.0, 33.1	0.0, 91.5	0.0, 91.5
Baseline ABR (treated bleeds), %[a,b]				
0 bleeds/year	7 (31.8)	36 (32.1)	43 (32.6)	43 (32.1)
> 0 to 4	9 (40.9)	33 (29.5)	41 (31.1)	32 (31.3)
> 4 to 10	1 (4.5)	28 (25.0)	28 (21.2)	29 (21.6)
> 10	5 (22.7)	15 (13.4)	20 (15.2)	20 (14.9)
Baseline ABR (all bleeds), bleeds/year[b]				
Mean (SD)	9.09 (22.55)	5.36 (6.93)	6.00 (11.14)	5.97 (11.06)
Median	1.38	3.28	2.74	2.79
Min, Max	0.0, 104.6	0.0, 34.6	0.0, 104.6	0.0, 104.6
Baseline ABR (all bleeds), %[a,b]				
0 bleeds/year	7 (31.8)	34 (30.4)	41 (31.1)	41 (30.6)
> 0 to 4	9 (40.9)	31 (27.7)	39 (29.5)	40 (29.9)
> 4 to 10	1 (4.5)	30 (26.8)	30 (22.7)	31 (23.1)
> 10	5 (22.7)	17 (15.2)	22 (16.7)	22 (16.4)
History of FVIII inhibitors, n (%)[a]				
Yes	0	1 (0.9)	1 (0.8)	1 (0.7)
No	22 (100)	111 (99.1)	131 (99.2)	133 (99.3)
History of previous diseases, n (%)[a]				

Characteristic	Directly Enrolled (N=22)	Rollover (N=112)	mITT (N=132)	ITT (N=134)
Hepatitis B	3 (13.6)	17 (15.2)	18 (13.6)	20 (14.9)
Hepatitis C	8 (36.4)	33 (29.5)	39 (29.5)	41 (30.6)
HIV	2 (9.1)	0	0	2 (1.5)
Number of target joints, n (%)[a]				
0	15 (68.2)	82 (73.2)	95 (72.0)	97 (72.4)
1	4 (18.2)	13 (11.6)	17 (12.9)	17 (12.7)
2	0	9 (8.0)	9 (6.8)	9 (6.7)
3	2 (9.1)	6 (5.4)	8 (6.1)	8 (6.0)
>3	1 (4.5)	2 (1.8)	3 (2.3)	3 (2.2)

ITT, intent-to-treat; mITT, modified intent-to-treat; ABR, annualized bleeding rate; FVIII, coagulation factor VIII; HIV, human immunodeficiency virus; IU, international unit; Max, maximum; Min, minimum; SD, standard deviation.

FVIII activity levels below the LLOQ (Lower limit of quantification) were imputed with 0 IU/dL.

[a] Percentages were calculated using the total number of subjects (N) in each analysis population as the denominator.

[b] For subjects in the Rollover Population, Baseline period was from Day 1 visit in 270-902 up to the BMN 270 infusion in 270-301 (approximately 6 months).

At least one medical history finding was reported for 124/134 subjects (92.5%) in the ITT Population, including 103/112 (92.0%) in the Rollover Population and 21/22 (95.5%) in the Directly Enrolled Population. The most commonly reported medical history findings by SOC in the ITT Population were Musculoskeletal and Connective Tissue Disorders (94 subjects [70.1%]); Surgical and Medical Procedures (78 subjects [58.2%]); and Infections and Infestations (53 subjects [39.6%]). The most commonly reported medical history findings by PT in the ITT Population were haemophilic arthropathy (45 subjects [33.6%]), hepatitis C (39 subjects [29.1%]), arthropathy (31 subjects [23.1%]), hepatitis B (14 subjects [10.4%]), and synoviorthesis (14 subjects [10.4%]). Incidence of preferred terms reported as medical history was similar between the Rollover Population and the Directly Enrolled Population.

It was anticipated that all subjects had a history of taking FVIII replacement therapy of some kind.

- **Numbers analysed**

The following populations have been defined:

- **Intent-to-Treat (ITT) Population (n=134)** – all subjects dosed in 270-301, used for the primary safety analyses.
- **Modified Intent-to-Treat (mITT) Population (n=132)** – all HIV-negative subjects dosed in 270-301, used for the primary analysis of the primary efficacy endpoint and the tertiary efficacy endpoints.
- **Rollover Population (n=112)** – all subjects dosed in 270-301 who previously participated in 270-902 (all subjects were HIV-negative), used for the primary analysis of the secondary efficacy endpoints.
- **Directly Enrolled Population (n=22)** – all subjects dosed in 270-301 who did not previously participate in 270-902
- **Directly Enrolled HIV-Negative Population (n=20)** – all HIV-negative subjects dosed in 270-301 who did not previously participate in 270-902

Despite the primary analysis of the primary efficacy endpoint was initially planned to use the mITT population, it was requested in the remaining clinical major objection from the withdrawn application to provide the primary analysis at week 52 on the full study cohort of 134 subjects. Indeed, acknowledging on one hand the medical need in HIV-infected patients who should not be deprived of this gene therapy, and on the other hand the hepatotoxicity of combined ARV treatments, it was agreed upon that HIV-infected patients could be treated with BMN 270.

- **Outcomes and estimation**

- ***Primary endpoint:*** *Change of the FVIII activity, as measured by chromogenic assay, from baseline to Week 49-52 post-BMN 270 infusion*

Table 14 Summary of Median FVIII Activity Level During Weeks 49-52 Using Chromogenic Substrate Assay (mITT and ITT Populations)

	Directly Enrolled (N=22)	Rollover (N=112)	mITT (N=132)	ITT (N=134)
Week 49-52 Median FVIII activity level (IU/dL)				
n	22	112	132	134
Mean (SD)	36.64 (45.82)	43.62 (45.32)	42.89 (45.51)	42.48 (45.31)
Median	23.53	24.15	23.92	23.92
Min, Max	1.6, 207.4	0.0, 231.2	0.0, 231.2	0.0, 231.2
Week 49-52 Median FVIII activity level change from baseline (IU/dL)*				
n	22	112	132	134
Mean (SD)	35.64 (45.82)	42.62 (45.32)	41.89 (45.51)	41.48 (45.31)
Median	22.53	23.15	22.92	22.92
Min, Max	0.6, 206.4	-1.0, 230.2	-1.0, 230.2	-1.0, 230.2
p-value	0.0015	< 0.0001	< 0.0001	< 0.0001
95% CI	15.32, 55.96	34.14, 51.11	34.05, 49.73	33.74, 49.22
Week 49-52 Median FVIII activity level, n(%)				
< 5 IU/dL	**3 (13.6)**	**13 (11.6)**	**16 (12.1)**	**16 (11.9)**
BLOQ (< 3.0 IU/dL)	2 (9.1)	10 (8.9)	12 (9.1)	12 (9.0)
≥ 3 - < 5 IU/dL	1 (4.5)	3 (2.7)	4 (3.0)	4 (3.0)
≥ 5 - < 40 IU/dL	**13 (59.1)**	**55 (49.1)**	**66 (50.0)**	**68 (50.7)**
≥ 5 - < 15 IU/dL	5 (22.7)	18 (16.1)	22 (16.7)	23 (17.2)
≥ 15 - < 40 IU/dL	8 (36.4)	37 (33.0)	44 (33.3)	45 (33.6)
≥ 40 IU/dL	**6 (27.3)**	**44 (39.3)**	**50 (37.9)**	**50 (37.5)**
≥ 40 - < 150 IU/dL	5 (22.7)	38 (33.9)	43 (32.6)	43 (32.1)
> 150 IU/dL	1 (4.5)	6 (5.4)	7 (5.3)	7 (5.2)

ITT, intent-to-treat; mITT, modified intent-to-treat; FVIII, Coagulation factor VIII; Max, maximum; Min, minimum; SD, standard deviation; CI, confidence interval.

Values for FVIII activity were excluded from analysis if obtained within 72 hours (or 3 calendar days if time is not available) since the last infusion of exogenous FVIII replacement therapy. In addition, post-baseline FVIII activity values were excluded if obtained after FVIII prophylaxis treatment resumed. FVIII activity levels below the LLOQ (Lower limit of quantification, 3.0 IU/dL for the chromogenic assay) were imputed with 0 IU/dL. If no assessments were available during weeks 49-52, the last observation carried forward (LOCF) approach was used to impute the missing value, specifically, the median value in the subject's last 4-week window containing a valid observation during weeks 5-48 was used to impute the missing value. If no such assessments were available, 1 IU/dL was used for imputation.

p-value is based on two-sided t-test against 0.

* Baseline FVIII activity value of 1 IU/dL was used in change from baseline analysis since there was no washout of severe hemophilia A subjects' usual FVIII prophylaxis (in order to avoid increasing the risk of bleeding) prior to BMN 270 infusion.

At Weeks 49-52 post-infusion, the median FVIII activity level (chromogenic assay) for the ITT Population was 23.92 IU/dL and mean (SD) FVIII activity level was 42.48 (45.31) IU/dL; similar results were found for the mITT population including only HIV-negative subjects. The FVIII activity levels at Weeks 49-52 post-infusion are similar to FVIII level of mild haemophilia (5-<40 IU/dL).

The median [min, max] time to peak FVIII activity using the chromogenic assay was **25.7 [2.00, 69.3] weeks** and **25.9 [2.00, 69.3] weeks** for the ITT and mITT Populations, respectively.

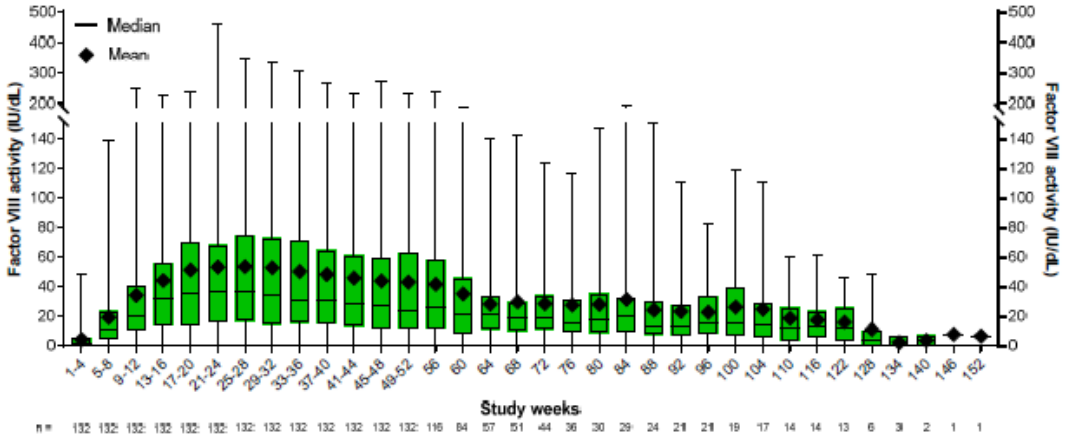
The mean (SD) and median [min, max] peak FVIII activity using the chromogenic assay were **84.5 (81.9)** and **61.2 [4.00, 463] IU/dL** for the ITT Population, respectively. The mean (SD) and median [min, max] peak FVIII activity using the chromogenic assay were 85.0 (82.5) and 61.2 [4.00, 463] IU/dL for the mITT Population, respectively.

Following peak FVIII activity, FVIII activity levels had a slow decline that decreased over time, and remained at a hemostatic level over the duration of the Week 52 analysis, i.e. median and mean (SD) FVIII activity level (chromogenic assay) for the ITT Population at 38.1 UI/dL and 52.6 (54.8) UI/dL at Week 23-26 (N=134), 23.92 IU/dL and 42.48 (45.31) IU/dL at Week 49-52 (N=134) and 13.9 UI/dL and 22.4 (28.8) UI/dL at Week 104 (N=19) respectively. The durability of the effect remains unpredictable since it is still unknown if the FVIII activity decrease might be sufficient to support hemostatic efficacy for multiple years or decline below the threshold of 5 IU/dL or return to baseline value. Moreover, the initial FVIII activity level decline is expected to vary among the treated patients providing that it is apparently proportionate to peak FVIII expression. Only a very long-term follow-up will help to elucidate this point; 5-year data will be provided in the framework of the Conditional marketing authorisation. While waiting for this data, the applicant provided the primary analysis on ITT population (N=134) at Week 104 to further characterize the treatment effect.

Table 15 Summary of FVIII Activity Level at Weeks 104 and 156 Using Chromogenic Assay

	Directly Enrolled (N=22)	270-902 Rollover (N=112)	mITT (N=132)	ITT (N=134)
Week 104 FVIII activity level (IU/dL)				
n	22	112	132	134
Mean (SD)	20.86 (27.04)	23.11 (33.90)	23.04 (32.94)	22.74 (32.79)
Median	11.40	11.65	11.75	11.65
Min, Max	0.0, 110.6	0.0, 187.1	0.0, 187.1	0.0, 187.1
Week 104 FVIII activity level, n (%)				
< 5 IU/dL	5 (22.7%)	28 (25.0%)	32 (24.2%)	33 (24.6%)
BLOQ (< 3.0 IU/dL)	4 (18.2%)	16 (14.3%)	19 (14.4%)	20 (14.9%)
≥ 3 - < 5 IU/dL	1 (4.5%)	12 (10.7%)	13 (9.8%)	13 (9.7%)
≥ 5 - < 40 IU/dL	14 (63.6%)	67 (59.8%)	80 (60.6%)	81 (60.4%)
≥ 5 - < 15 IU/dL	8 (36.4%)	38 (33.9%)	45 (34.1%)	46 (34.3%)
≥ 15 - < 40 IU/dL	6 (27.3%)	29 (25.9%)	35 (26.5%)	35 (26.1%)
≥ 40 IU/dL	3 (13.6%)	17 (15.2%)	20 (15.2%)	20 (14.9%)
Week 156 FVIII activity level (IU/dL)				
n	19		17	19
Mean (SD)	15.22 (20.44)		16.8 (21.08)	15.22 (20.44)
Median	8.40		9.3	8.40
Min, Max	0.0, 62.2		0.0, 62.2	0.0, 62.2
Week 156 FVIII activity level, n (%)				
< 5 IU/dL	7 (36.8)		5 (29.4)	7 (36.8)
BLOQ (< 3.0 IU/dL)	5 (26.3)		4 (23.5)	5 (26.3)
≥ 3 - < 5 IU/dL	2 (10.5)		1 (5.9)	2 (10.5)
≥ 5 - < 40 IU/dL	9 (47.4)		9 (52.9)	9 (47.4)
≥ 5 - < 15 IU/dL	8 (42.1)		8 (47.1)	8 (42.1)
≥ 15 - < 40 IU/dL	1 (5.3)		1 (5.9)	1 (5.3)
≥ 40 IU/dL	3 (15.8)		3 (17.6)	3 (15.8)

Figure 8 Box Plot for Median Factor VIII Activity Level Using Chromogenic Substrate Assay by 4-Week and 6-Week Windows (mITT Population)



Baseline value is the most recent FVIII activity prior to BMN 270 infusion while on FVIII prophylaxis. Post-baseline values for FVIII activity were excluded from analysis if obtained within 72 hours (or 3 calendar days if time is not available) since the last infusion of exogenous FVIII replacement therapy. FVIII activity levels below the LLOQ (Lower limit of quantification, < 3 IU/dL for the chromogenic assay) were imputed with 0 IU/dL.

The boxes show the interquartile ranges with the lines in the boxes indicating medians. The ends of the whiskers represent the minimum and maximum values and diamonds indicate the means.

A total of 16/132 subjects (**12.1%**) had a median **FVIII activity level <5 IU/dL** at Weeks **49-52** corresponding to the FVIII level limit of moderate haemophilia and to subjects who do not respond to BMN 270 treatment as define in the primary endpoint.

When examining the trajectory of FVIII activity over time until Week 156, the tri-phasic pattern seen in the 270-201 study is recapitulated in Study 270-301, with an increase in FVIII activity over the first 6 months after BMN 270 infusion, followed by a decline in FVIII activity and a subsequent flattening of the slope around 18 months after infusion.

Figure 9 FVIII Activity Trajectory in Phase 3 Parallels Phase 1/2 (Chromogenic Assay) Over Time: Mean (±SE)

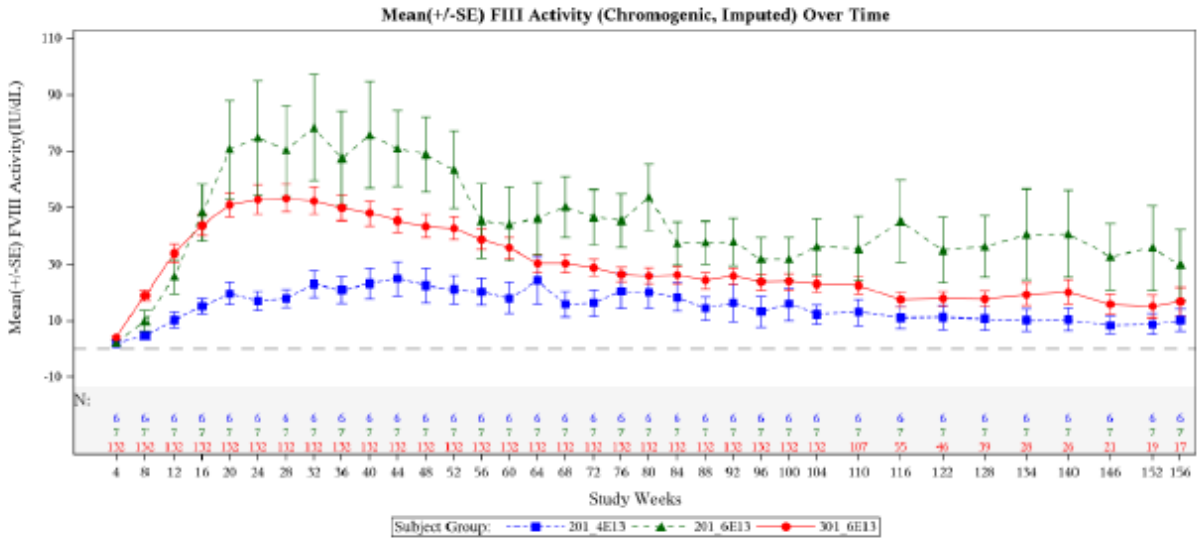


Table 16 FVIII activity level <5 UI/dL at Weeks 49-52 in Study 270-301

Week 49-52	**Baseline**		**From Week 5 to last visit**		**Peak**		**Data cutoff**	
Median FVIII activity (UI/dL)	ABR fir Treated bleeds (bleeds/year)	Annualised FVIII usage (IU/kg/year)	ABR for Treated bleeds (bleeds/year)	Annualised FVIII usage (IU/kg/year) (% change from baseline)	Peak FVIII activity (UI/dL)	Timing of Peak	Follow-up (weeks)	Final FVIII activity (UI/dL)
<3.0	2	1760	0	22 (98,8%)	27.4	Week 25	60.1	<3.0
1,55	17,6	7838,2	1,2	146,8 (98,1%)	30,8	Week 12	140,4	<3.0
1,55	0	2550,9	0 (but 2 bleeding events at Day 176 and Day 455 treated outside the window)	72,7 (97,1%)	4	Week 8	66,1	<3.0
<3.0	4,9	1361,6	27,3	1063,3 (21,9%)	14,3	Week 14	60	<3.0
3.5 (imputed)	12.3	5022.8	7.9	849.6 (83.1%)	10.3	Week 4	102.1	1
3,7	19,2	5394,1	4,6	1510,5 (72%)	4,8	Week 40 and	61,1	3,9

						Week 50		
<3,0	22	5025,2	6,1	344,5 (93,1%)	19,2	Week 16	56,3	3,2
<3,0	2,1	3617,9	10,4	322,8 (91,1%)	4,5	Week 19	59,7	<3.0
4,55	3,8	3363,8	0	0 (100%)	8,8	Week 38	56,1	4,9
3,2	0	6483,7	0	72,8 (98,9%)	24,9	Week 13	61,1	<3,0
<3,0	3,8	2684,5	0	0 (100%)	8,4	Week 28	55,1	<3,0
<3,0	3,2	1755,9	0,8	94,1 (94,6%)	27,6	Week 17	71,9	<3,0
<3,0	9,9	2089,2	0	53,4 (97,4%)	75	Week 38	60,4	<3,0
2,95	11,1	2142,6	0	17,7 (99,2%)	5,9	Week 51	52,1	<3,0
<3,0	0	4065,3	0,9	36,7 (99,1%)	7,6	Week 31	62,1	4,6
2,05	11,7	4318,9	6,4	719,1 (83,3%)	6,7	Week 36	69,9	3,1

Source: Integrated Subject Narratives, 270-301 Individual Subject Clinical Efficacy Report

For both non-responders, a peak FVIII activity was reached after the BMN 270 administration based on the provided narratives. For the large majority of these subjects (15/16, 93.8%), the peak remained lower than the median peak FVIII activity using the chromogenic assay of 61.2 IU/dL. Two subjects restarted a continuous prophylaxis after one year post-BMN 270 administration due to low FVIII levels; one subject had FVIII replacement and reported no additional bleeding, one received emicizumab with no noticeable ABR improvement.

Of the 134 subjects in the ITT Population, 132 (98.5%) remained off FVIII prophylaxis at the first data cutoff providing one year of follow-up (Nov 16, 2020) after a single BMN 270 infusion. Two subjects restarted continuous FVIII prophylaxis following BMN 270 infusion:

- Subject

His baseline ABR (treated bleeds) was 12.3 bleeds/year, and his baseline annualised FVIII usage was 5022.8 IU/kg/year, given over 144.8 infusions/year. No target joints were identified.

Following BMN 270 infusion, the subject remained on continuous FVIII prophylaxis until Day 24. His FVIII activity by chromogenic assay on Day 28 was 10.3 IU/dL (9.4 IU/dL by one-stage assay), but it declined to < 3.0 IU/dL at Day 43, Day 50, Day 55, and Day 66 (onestage assay FVIII activity during that period ranged from 1.8-4.1 IU/dL). He continued to receive Kogenate 3000 IU prophylactic infusions during this period, approximately once every 5-6 days, and he was also started on oral

corticosteroids (prednisolone 60 mg/day starting on Day 44) in light of his decreased FVIII activity (his ALT levels remained within normal limits throughout the study).

On Day 70, FVIII prophylaxis was stopped completely. His FVIII activity by chromogenic assay on that day was 5.4 IU/dL (but was drawn within 72 hours of a FVIII infusion). The corticosteroid regimen was tapered starting at Day 67, and completed at Day 116. Between Day 70 and Day 142, his FVIII activity by chromogenic factor peaked at 3.2 IU/dL (Day 104); all other assessments were < 3.0 IU/dL. He resumed periodic prophylactic FVIII infusions again on Day 158, in addition to multiple infusions for concurrent bleeding events. His FVIII activity level at Day 401 was < 3.0 IU/dL (chromogenic) and 1.7 IU/dL (one-stage).

On Day 406, the subject resumed continuous prophylaxis with emicizumab (525 mg/month), and remained on emicizumab as of the data cutoff (Day 952). Following the start of emicizumab prophylaxis, the subject continued to receive periodic FVIII infusions as prophylaxis (5 times between Day 406 and 418, and then 7 in total between Day 452 and Day 593 ranging from 8-44 days apart), as well as FVIII infusions as bleeding event treatment. As of the data cutoff, the subject had completed 136 weeks of post-infusion follow-up.

The subject had an ABR for treated bleeds from Week 5 to the last visit of 7.9 bleeds/year.

The subject's ABR from Week 5 to the resumption of continuous prophylaxis with emicizumab was 11.8 bleeds/year for treated bleeds, and 12.7 bleeds/year for all bleeds. His ABR from the start of emicizumab prophylaxis to the data cutoff was 12.1 bleeds/year for treated bleeds and overall (no untreated bleeds were reported during this period). Starting at Week 5, he recorded 24 bleeding events.

- Subject

At Weeks 49-52, the subject's median FVIII activity by chromogenic assay was 2.05 IU/dL. His FVIII activity peaked at 6.7 IU/dL (chromogenic assay) at Week 36.

Following BMN 270 infusion, the subject remained on continuous FVIII prophylaxis until Day 28. His FVIII activity by chromogenic assay on Day 28 was < 3.0 IU/dL (1 IU/dL by one-stage assay). Between Day 28 and Day 393, his FVIII activity ranged from < 3.0 – 6.7 IU/dL and from 2.6-12.3 IU/dL (one-stage; peak at Day 307). As of the data cutoff, the subject had completed 69.9 weeks of post-infusion follow-up; his final FVIII assessment by chromogenic assay prior to the data cutoff was 3.1 IU/dL on Day 393.

The subject had an ABR for treated bleeds from Week 5 to the last visit of 6.4 bleeds/year. Starting at Week 5, he recorded 8 bleeding events. In addition, he underwent a liver biopsy at Day 244 and received Alphanate 15806 IU, divided over 6 infusions (Day 244-247). The subject also received Alphanate as one-time FVIII prophylaxis on Day 65, Day 178, and Day 228. He resumed continuous FVIII prophylaxis with Alphanate on Day 394. No additional bleeding events were reported following the resumption of FVIII continuous prophylaxis.

Despite their low median FVIII activity level at Weeks 49-52, 62.5% (10/16) of subjects with FVIII activity level < 5 IU/dL at Week 52 gained an improved ABR for treated bleeds from Week 5 to last visit. Two of the 16 subjects (12.5%) remained at ABR 0, the impact of BMN 270 remaining uncertain. Also significant reductions in the use of exogenous FVIII is observed in the 16 subjects having a median FVIII activity level <5 IU/dL at Weeks 49-52; BMN 270 reduced annualised FVIII utilisation by ≥90% in 75% (12/16) of these subjects.

One subject experienced a worsening of the control of his disease. Subject having FVIII activity level <3 IU/dL at Weeks 49-52 had an abnormal ABR high value at Week 5 to beyond (from 4.9 bleeds/year at baseline to 27.3 treated bleeds/year from Week 5 to beyond) and a limited improvement of 21.9%

of annualised FVIII usage between baseline and Week 5 to last visit. His follow-up was 60 weeks; an updated narrative at 111 weeks follow-up was provided. Low FVIII activity levels were recorded with levels < 3.0 IU/dL through Week 60 and < 1.5 IU/dL from Week 64 to the data cutoff, with the exception of 3 assessments between 1.6-2.3 IU/dL at Week 64, Week 72, and Week 104. His ABR from the end of FVIII prophylaxis at Week 9 to Week 111 was still higher than the baseline ABR value (16.7 bleeds/year). Based on the narrative, the subject denied any problems with functioning of his elbows, showed no interest in seeing an orthopedist for further evaluation or consideration of treatment such as corticosteroid injections, and did not want to resume FVIII prophylaxis, which could prevent clinical improvement.

Clinically relevant effects can be observed in subjects with a median FVIII activity level <5 IU/dL from Week 5 to beyond, with a mean follow-up period of 66.2 weeks (approximately 1 year and 3 months) but remain unpredictable.

As of the **2-year data cut** (15 November 2021) for study 270-301, 33 of 134 (**24.6%**) ITT population and 28 of 112 (**25%**) 270-902 rollover subjects had **FVIII activity < 5 IU/dL** as measured by CSA at Week 104. Most subjects with FVIII activity < 5 IU/dL have a good bleeding control, with 14 having an ABR of zero, 8 having an ABR < 1 and 11 having an ABR > 1. Only 2 of 11 subjects with ABR >1 had a higher ABR post BMN 270 infusion compared to baseline. The annualised bleeding rate (ABR) post FVIII prophylaxis period in the 28 of 112 Rollover subjects from study 270- 902 with FVIII activity <5 IU/dL was 2.21 (4.38) and 13 of 28 (46.4%) subjects had zero bleeds at week 104.

Of the subjects that had FVIII activity < 5 IU/dL at the 2-year data cut (15 November 2021), 5 have returned to routine prophylaxis (4 subjects on FVIII replacement therapy and 1 on emicizumab) not including the 2 subjects with higher ABR post BMN 270 infusion compared to baseline. One additional subject in 270-301 study at the 2-year data cut has also returned to FVIII prophylaxis although his median Week 104 FVIII activity was 5.5 IU/dL per chromogenic assay. This subject has had multiple traumatic bleeds due to participation in sports and has had multiple FVIII activity levels between 3-5 IU/dL.

The status of responder/non-responder to BMN 270 remains unexplained and unpredictable with a wide variability of the peak FVIII activity and time to peak FVIII activity observed among the non-responder subjects and an unpredictable clinical response.

Variability on FVIII activity levels

There was a wide range of FVIII activity during Week 49-52 reflecting a high variability of FVIII levels among the treated-subjects, i.e. from 0 to 231.2 UI/dL in ITT population as measured by the chromogenic assay. Extra high levels of FVIII activity (>150 IU/dL) can lead to management issues in case of durability of such levels and were reported in 7/134 (5.2%) patients at Weeks 49-52. A total of 15/134 (11.2%) subjects had FVIII activity level > 250 IU/dl at one or more timepoints. The updated narrative of Subject reports FVIII levels variations including increases from Week 60, an extra high FVIII activity between Weeks 60 and 90 and the persistence of high level of FVIII activity on his final assessment on Day 730 (110.6 IU/dL (chromogenic)/146.7 IU/dL (one-stage)). According to the narratives, none of these subjects report any thromboembolic adverse events and subjects with history of arterial or venous thromboembolic events were excluded. A special warning in patients with history of arterial or venous thromboembolic events is proposed in section 4.4 of the SmPC. The applicant further investigated the potential impact of durability of FVIII activity levels > 150 IU/dL and no thromboembolic event was observed in Study 270-301 as of the 2-year data cut which do not suggest a potential impact of the durability of supra-physiologic FVIII activity levels.

- *Impact of the use of corticosteroids on FVIII activity*

The impact of the use of corticosteroids was evaluated between the Directly enrolled (N=22) and the Rollover populations (N=112) in Study 270-301.

The ALT elevation warranting consideration of corticosteroid treatment was modified several times:

- At the start of study, it was defined as ALT ≥1.5x ULN.
- Protocol Amendment 3, it was defined as ALT > 2x baseline and > ULN.
- Protocol Amendment 6, it was lowered to ALT ≥ ULN or ≥ 1.5 x baseline ALT.

The timing of these changes in corticosteroid use led to differences in how corticosteroids were used in the Directly Enrolled and Rollover Populations:

- Directly Enrolled Population (N=22) (Subjects enrolling earlier in 270-301) generally initiated corticosteroids reactively if ALT ≥ 1.5x the ULN, and later amended to initiate reactive corticosteroids if ALT ≥ 1.5x ULN or ALT > ULN and >2x baseline ALT.
- Rollover Population (N=112) (Subjects enrolling later) generally initiated corticosteroids at the lower ALT threshold of ALT > ULN or ≥1.5 x baseline ALT. In addition, reactive corticosteroids were tapered only after ALT levels returned to baseline levels, which effectively prolonged both the total dose and duration of corticosteroids administered.

The time from BMN 270 infusion to first corticosteroid use was similar across analysis populations, with mean (SD) time of 9.9 (4.2), 11.0 (10.4), and 10.9 (9.8) weeks in the Directly Enrolled, Rollover and ITT Populations, respectively.

Total duration of corticosteroid use was longer in the Rollover compared to Directly Enrolled population with a mean (SD) duration of **242.9 (113.3) days** compared to **179.1 (122.9**). The longer duration of use in the Rollover Population also led to a higher total corticosteroid dose in the Rollover Population with a mean (SD) total dose of **9279 (6605) mg** compared to **4659 (2439) mg** in the Directly Enrolled population.

For the Rollover Population, the mean (SD) change from baseline at Weeks 49-52 of median FVIII activity was **42.62 (45.32) IU/dL**, with a median change from baseline of **23.15 IU/dL**. Out of 112 Rollover Population subjects, 99 (88.4%) had median FVIII activity levels in the mild or non-haemophilic range.

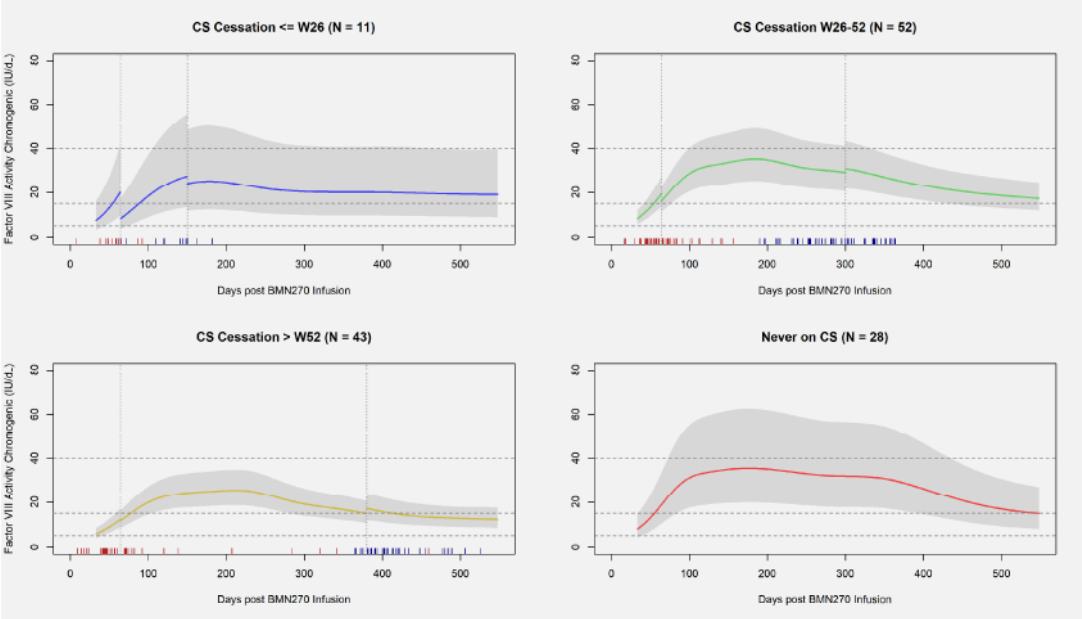
Subjects in the Directly Enrolled Population had a mean (SD) change from baseline at Weeks 49-52 of **35.64 (45.82) IU/dL**, with a median change of **22.53 IU/dL**. Similar to other populations, 86.4% of Directly Enrolled subjects (19/22) had median FVIII activity levels in the mild or non-haemophilic range.

Despite a longer mean duration of corticosteroids use and an almost two times higher total corticosteroids dose in the Rollover population compared to the Directly enrolled population, the median FVIII activity was similar between these 2 populations. It is noted that the time from BMN 270 infusion to first corticosteroid use was similar across the 2 populations (mean (SD) time of 9.9 (4.2) and 11.0 (10.4) weeks in Directly Enrolled and Rollover population, respectively). Since the corticosteroids uses differed on duration of the use and the total dose between the two populations compared, the results suggested that these parameters are likely not impact the FVIII activity.

Post-hoc analyses of longitudinally collected FVIII activity levels, corticosteroid usage and ALT of subjects in the ITT Population was conducted using mixed effects models to further evaluate the potential impacts of the initiation and cessation of corticosteroids on FVIII expression. After controlling for the nonlinear time effect and ALT level prior to each FVIII measure as covariates, no significant

change in the FVIII activity level was found after corticosteroids cessation, compared to the FVIII level measured when corticosteroids were in use, including for the subjects who were on corticosteroids for longer duration. Figure 10 shows model estimated FVIII trajectories for subjects whose corticosteroids cessation was before Week 26 (n=11), between Weeks 26-52 (n=52), after Week 52 (n=43), and who did not take corticosteroids before data cut (n=28). The two discontinuities of each FVIII curve (except the last subgroup) represent the estimated effects of the initiation and cessation of corticosteroids (denoted by the two vertical lines), respectively. The slight decline (cessation before Week 26) or increase (cessation after Week 26) in FVIII level before and after corticosteroids cessation are not statistically significant based on this modeling approach

Figure 10 Mean FVIII Activity Level Over Time Estimated by Mixed Effects Models following Cessation of Corticosteroids or for Patients Who Did Not Take Corticosteroids



The comparison of the median FVIII activity before and after immunosuppressant discontinuation does not highlight a clear difference in median FVIII activity that could not be imputed to the observed decrease of FVIII activity over the time.

- ***First secondary endpoint:*** *Change in the annualised utilisation (IU/kg) of exogenous FVIII replacement therapy during Week 5 - Last Visit by data cutoff post-BMN 270 infusion from baseline*

At DCO date of 16 November 2020

A clear decrease of FVIII use is observed in the Rollover population (N=112) with a decline of 98.6% of the annualised FVIII utilisation from baseline to Week 5 to last visit. Similar results were observed in ITT and mITT populations. The main reasons of FVIII infusion was first the treatment for a bleeding episode then routine FVIII prophylaxis for 3 patients.

Table 17 Summary of Annual*ised* Util*isation* of Exogenous FVIII Replacement Therapy (mITT and ITT Populations)

Cohort	Baseline	Post-Baseline				
		Weeks 1-4		Week 5 to Data Cutoff		
	Annualized FVIII Utilization (IU/kg/yr)	FVIII Utilization (IU/kg)	Annualized FVIII Utilization (IU/kg/yr)	FVIII Utilization (IU/kg)	Annualized FVIII Utilization (IU/kg/yr)	Change from Baseline (IU/kg/yr)
Rollover Population (N=112)						
Mean (SD)	3961.17 (1751.47)	268.50 (130.95)	3064.72 (1494.64)	63.07 (213.62)	56.89 (194.58)	-3904.29 (1755.53) 98.6%
Median	3754.42	241.62	2757.89	0.00	0.00	-3716.64
Min, Max	1296.4, 11251.1	43.1, 816.3	492.0, 9317.6	0.0, 1637.7	0.0, 1510.5	-11251.1, -298.3
ITT Population (n=134)						
Mean (SD)	4113.52 (1738.96)	273.15 (132.14)	3117.72 (1508.26)	110.58 (329.22)	73.20 (208.05)	4040.32 (1731.98) 98.2%
Median	3860.30	250.17	2855.50	0.00	0.00	-3811.47
Min, Max	1296.4, 11251.1	40.9, 816.3	466.5, 9317.6	0.0, 2140.1	0.0, 1510.5	-11251.1, -298.3
mITT Population (n=132)						
Mean (SD)	4111.30 (1747.82)	271.89 (132.09)	3103.33 (1507.74)	96.18 (285.21)	67.26 (196.93)	-4044.04 (1743.50) 98.4%
Median	3860.30	249.53	2848.19	0.00	0.00	-3811.47
Min, Max	1296.4, 11251.1	40.9, 816.3	466.5, 9317.6	0.0, 2140.1	0.0, 1510.5	-11251.1, -298.3
Directly Enrolled Population (n=22)						
Mean (SD)	4889.11 (1477.54)	296.79 (138.75)	3387.53 (1583.69)	352.44 (609.86)	156.23 (255.69)	-4732.87 (1451.68) 96.8%
Median	4785.87	292.16	3334.69	83.06	42.08	-4440.77
Min, Max	2550.9, 7885.0	40.9, 608.7	466.5, 6947.7	0.0, 2140.1	0.0, 892.2	-7875.4, -2478.3

Annualized FVIII utilization (IU/kg/yr) = (sum(FVIII replacement treatment (IU/kg) of the period)/sum(follow-up days of the period))*365.

In the 270-301 ITT Population, 59.7% (80/134) of subjects have required no FVIII infusions after Week 5; that proportion increases to 67% (75/112) for the Rollover Population.

In total, the 34 subjects in the Rollover Population who had FVIII utilisation from Week 5 to the last visit as of the data cutoff received a total of 243 FVIII infusions.

Of these 243 FVIII infusions, 109 (44.9%) were given as treatment for a bleeding episode, 15.6% were given as treatment for a surgery/procedure, and 9.5% were given as one-time FVIII prophylaxis. An additional 30.0% were given as routine FVIII prophylaxis; while the 270-301 protocol called for all subjects to discontinue routine FVIII prophylaxis by Week 4, 3 subjects remained on routine FVIII prophylaxis for 10 weeks or more following BMN 270 infusion (range 10.0-16.7 weeks) at the Investigator's discretion due to slow FVIII activity level response to BMN 270.

At DCO date of 15 November 2021

Clinical data from the 2-year data cut continue to demonstrate virtual elimination of the use of exogenous FVIII, with statistically significant reductions in annualised exogenous FVIII to treat bleeding episodes through Week 104. A single dose of BMN 270 significantly reduced mean annualised FVIII utilisation (AFU) in the Rollover Population by 98.2% from a mean (SD) of 3961.17 (1751.47;

median 3754.42) IU/kg/year to a mean (SD) of 69.90 (209.22; median 0.00) IU/kg/year (p-value <0.0001).

Table 18 Summary of Annualised Utilisation (IU/kg/yr) of Exogenous FVIII Replacement Therapy

Cohort	Baseline	Efficacy Evaluation Period*		
	Annualised FVIII Utilization (IU/kg/yr)	FVIII Utilization (IU/kg)	Annualised FVIII Utilization (IU/kg/yr)	Change from Baseline (IU/kg/yr)
Rollover Population (N=112)				
Mean (SD)	3961.17 (1751.47)	140.91 (423.00)	69.90 (209.22)	-3891.27 (1761.17)
Median	3754.42	0.00	0.00	-3740.41
Min, Max	1296.4, 11251.1	0.0, 3169.2	0.0, 1480.2	-11251.1, -752.0
ITT Population (n=134)				
Mean (SD)	4113.52 (1738.96)	205.17 (583.15)	85.39 (229.80)	-4028.31 (1735.80)
Median	3860.30	0.00	0.00	-3809.07
Min, Max	1296.4, 11251.1	0.0, 4310.9	0.0, 1480.2	-11251.1, -752.0
mITT Population (n=132)				
Mean (SD)	4111.30 (1747.82)	173.84 (464.30)	76.19 (205.09)	-4035.29 (1747.47)
Median	3860.30	0.00	0.00	-3809.07
Min, Max	1296.4, 11251.1	0.0, 3169.2	0.0, 1480.2	-11251.1, -752.0
Directly Enrolled Population (n=22)				
Mean (SD)	4889.11 (1477.54)	532.30 (1036.26)	164.23 (308.70)	-4725.94 (1441.40)
Median	4785.87	90.06	32.46	-4540.58
Min, Max	2550.9, 7885.0	0.0, 4310.9	0.0, 1311.0	-7885.0, -2478.3

* from Week 5 post-BMN 270 dosing (Study Day 33) or 3 days after the end of FVIII prophylaxis to the last visit prior to the data cut

- ***Second secondary endpoint: Annualised bleeding rate (ABR)***

  Change in the annualised number of bleeding episodes or requiring exogenous FVIII replacement treatment during Week 5 – Last Visit by data cutoff post-BMN 270 infusion from baseline

At DCO date of 16 November 2020

A significantly reduction of ABR for treated bleeds was observed in the Rollover population, i.e. a decline of 83.8% from mean baseline ABR to a mean ABR from Week 5 to the last visit as of the data cutoff.

Table 19 Summary of ABR (Bleeds Requiring Exogenous FVIII Replacement Treatment)

Cohort	Baseline	Post-Baseline				
		Weeks 1-4		Week 5 to Data Cutoff		
	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Change from Baseline (no/yr)
Rollover Population (n=112)						
Mean (SD)	4.83 (6.47)	0.15 (0.38)	1.73 (4.39)	0.83 (3.16)	0.78 (2.99)	-4.05 (6.93) 83.8%
Median	2.80	0.00	0.00	0.00	0.00	-1.87
Min, Max	0.0, 33.1	0.0, 2.0	0.0, 22.8	0.0, 29.0	0.0, 27.3	-33.1, 22.4
ITT (n=134)						
Mean (SD)	5.42 (9.96)	0.17 (0.47)	1.96 (5.33)	1.11 (3.49)	0.84 (2.84)	-4.58 (10.23) 84.5%
Median	2.30	0.00	0.00	0.00	0.00	-1.80
Min, Max	0.0, 91.5	0.0, 3.0	0.0, 34.2	0.0, 29.0	0.0, 27.3	-91.5, 22.4
mITT Population (n=132)						
Mean (SD)	5.43 (10.04)	0.17 (0.47)	1.99 (5.37)	1.05 (3.46)	0.82 (2.85)	-4.61 (10.31) 84.9%
Median	2.04	0.00	0.00	0.00	0.00	-1.78
Min, Max	0.0, 91.5	0.0, 3.0	0.0, 34.2	0.0, 29.0	0.0, 27.3	-91.5, 22.4
Directly Enrolled Population (n=22)						
Mean (SD)	8.41 (19.90)	0.27 (0.77)	3.11 (8.76)	2.55 (4.65)	1.13 (1.93)	-7.28 (20.00) 86.6%
Median	0.93	0.00	0.00	0.00	0.00	-0.93
Min, Max	0.0, 91.5	0.0, 3.0	0.0, 34.2	0.0, 20.0	0.0, 7.9	-91.5, 3.5

ITT, intent-to-treat; mITT, modified intent-to-treat; Max, maximum; Min, minimum; SD, standard deviation; ABR, annualized bleeding rate.

The annualized bleeding rate is defined as (Number of bleeding episodes during the calculation period / Total number of days during the calculation period) * 365.25.

Similar reductions in ABR for treated bleeds are seen in the ITT Population (84.5% reduction in ABR from Week 5 to the data cutoff). Of the 134 ITT Population subjects, 101 (75.4%) reported no bleeding episodes from Week 5 to the data cutoff, compared with 43 subjects (32.1%) during the pre-study historical period.

The majority of subjects in the Rollover population (79.5%) reported no treated bleeds from Week 5 to the last visit and a total of 7 subjects had more than 3 bleeds of which Subject that experienced the maximum value of number of bleeding episodes (29) and ABR (27.3 /year) from Week 5 to data cutoff and was a non-responder with median FVIII activity <3.0 IU/dL at Week 52.

At DCO date of 15 November 2021

Clinical data from the 2-year data cut demonstrate substantial reduction in bleeds. In the Rollover population, BMN 270 significantly reduced ABR for treated bleeds by 82.4% at year 1, 86.7% at year 2, and 84.5% over the efficacy evaluation period, all as compared to baseline. ABR was reduced from a prospectively collected mean (SD) baseline ABR of 4.83 (6.47) (median 2.80) treated bleeding episodes/year to a mean (SD) ABR of 0.75 (2.44) (median 0.0) treated bleeding episodes/year (p-value <0.0001) over the efficacy evaluation period.

Table 20 Summary of ABR (Bleeds Requiring Exogenous FVIII Replacement Treatment)

Cohort	Baseline	Efficacy Evaluation Period*		
	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Change from Baseline (no/yr)
Rollover Population (n=112)				
Mean (SD)	4.83 (6.47)	1.5 (4.9)	0.75 (2.44)	-4.08 (6.57)
Median	2.80	0.0	0.00	-2.30
Min, Max	0.0, 33.1	0, 34	0.0, 17.3	-33.1, 12.4
ITT (n=134)				
Mean (SD)	5.42 (9.96)	2.0 (6.0)	0.85 (2.52)	-4.57 (10.07)
Median	2.30	0.0	0.00	-1.80
Min, Max	0.0, 91.5	0, 42	0.0, 17.3	-91.5, 12.4
mITT Population (n=132)				
Mean (SD)	5.43 (10.04)	1.7 (4.9)	0.75 (2.31)	-4.68 (10.09)
Median	2.04	0.0	0.00	-1.82
Min, Max	0.0, 91.5	0, 34	0.0, 17.3	-91.5, 12.4
Directly Enrolled Population (n=22)				
Mean (SD)	8.41 (19.90)	4.5 (9.5)	1.37 (2.88)	-4.08 (6.57)
Median	0.93	0.0	0.00	-2.30
Min, Max	0.0, 91.5	0, 42	0.0, 17.3	-33.1, 12.4
Enrolled 3+ Years mITT (n=17)				
Mean (SD)	9.45 (22.49)	2.35 (4.91)	0.70 (1.45)	-8.75 (22.51)
Median	0.91	0.00	0.00	-0.91
Min, Max	0.0, 91.5	0.0, 19.0	0.00, 5.6	-91.5, 2.5

* from Week 5 post-BMN 270 dosing (Study Day 33) or 3 days after the end of FVIII prophylaxis to the last visit prior to the data cut

- ***Haemo-QoL-A***

HAEMO-QoL-A is a validated and specific quality of life questionnaire for patients with haemophilia (Rentz, Haemophilia 2008). The baseline scores were considered high despite the severe haemophilia, with a median total score of 80.05 in mITT population and 80.48 in ITT population which may be reflective of the 'disability paradox', i.e. patients with inherited and long-term conditions such as haemophilia have been shown to adapt to their levels of disability, often reporting better quality QoL than expected from the general population (O'Hara and co, 2021). A wide variability was however observed, i.e. 14.2-97.8 in both mITT and ITT populations. The Haemo-QoL-A score was improved from baseline to Week 52 with a mean and median change from baseline of 6.44 and 5.65 in both mITT and ITT populations.

At Week 104, there were clinically meaningful improvements in HRQoL (Quinn 2020), as assessed by the Haemo-QoL-A Total score (improvements at Weeks 52 [6.34 (11.99), p<.0001] and 104 [6.92 (12.54), <.0001] in 270-301) in the ITT population.

Summary of main efficacy results

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

Table 21 Summary of efficacy for trial 270-301

Title: A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII in Hemophilia A Patients with Residual FVIII Levels ⩽ 1 IU/dL Receiving Prophylactic FVIII Infusions			
Study identifier	270-301		
Design	Open-Label, Single-Arm, Multi-centre		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Approximately 264 weeks (for each subject) not applicable not applicable
Hypothesis	Superiority over baseline		
Treatments groups	**ITT population** (all subjects dosed)		Single infusion of BMN 270 6E13 vg/kg, Week 52 of post-infusion follow-up, N = 134
	Rollover population (all subjects dosed in 270-301 who previously participated in 270-902, all subjects were HIV-negative)		Single infusion of BMN 270 6E13 vg/kg, Week 52 of post-infusion follow-up, N = 112
Endpoints and definitions	Primary endpoint	FVIII activity	Change of hFVIII activity by chromogenic assay during Weeks 49-52 post-BMN 270 infusion from baseline
	Secondary endpoints	Exogenous FVIII Use	Change of the annualised utilisation (IU/kg) of exogenous FVIII replacement therapy during Week 5 to the last visit as of the data cutoff post-BMN 270 infusion from baseline
		ABR	Change in the annualised number of bleeding episodes requiring exogenous FVIII replacement treatment during Week 5 to the last visit as of the data cutoff post-BMN 270 infusion from baseline
Database lock	16 November 2020		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Primary endpoint: ITT population at Week 49-52 Secondary endpoints: Rollover population during Week 5 to Data cutoff		
Descriptive statistics and estimate variability	Treatment group	ITT	
	Number of subject	134	
	Week 49-52 Median FVIII activity level (IU/dL) (mean, median)	41.48, 22.92	
	Min, Max	-1.0, 230.2	
Analysis population and time point description	Rollover population Week 49-52		
	Treatment group	Rollover population	
	Number of subject	112	

Title: A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII in Hemophilia A Patients with Residual FVIII Levels ⩽ 1 IU/dL Receiving Prophylactic FVIII Infusions			
Study identifier	270-301		
	Annualised FVIII utilisation – Week 5 to data cutoff (IU/kg/yr) (mean, median)	56.89, 0.00	
	Min, Max	0.0, 1510.5	
	ABR - Week 5 to data cutoff (no/yr) (mean, median)	0.78, 0.00	
	Min, Max	0.0, 27.3	
Effect estimate per comparison	Annualised FVIII utilisation	Comparison groups	Rollover population
		Change from baseline (IU/kg/yr) (mean)	-3904.29 (98.6%)
		Min, Max	-11251.1, -298.3
	ABR	Comparison groups	Rollover population
		Change from baseline (no/yr) (mean)	-4.05 (83.8%)
		Min, Max	-33.1, 22.4
Notes	Due to several changes to statistical plan of which the baseline value imputed as 1 IU/dL not initially planned in the original protocol, only absolute values are reported in the table for the primary endpoint.		

2.6.5.3. Clinical studies in special populations

The efficacy results for the two HIV-positive subjects in 270-301 (1633-3005 and 1009-3002) are in-line with those in 270-201 6E13 vg/kg cohort and the 270-301 mITT Population, suggesting that HIV-positive patients stand to benefit clinically from BMN 270 as well in the absence of the use of highly potentially hepatotoxic concomitant medications to control their HIV infection.

Subject

His first FVIII activity assessment after prophylaxis was 10.8 IU/dL (chromogenic) on Day 37. His FVIII activity peaked at Week 12 (45.1 IU/dL). At Weeks 49-52, the subject's median FVIII activity by chromogenic assay was 6.1 IU/dL.

As of the data cutoff, the subject had completed 124 weeks of post-infusion follow-up; his final FVIII assessment by chromogenic assay prior to the data cutoff was < 3.0 IU/dL on Day 821.

The subject had an ABR for treated bleeds from Week 5 to the last visit of 3.5 bleeds/year.

Starting at Week 5, 19 bleeding events were reported, 8 of which required exogenous FVIII as treatment.

The subject also received extensive FVIII replacement as one-time prophylaxis (between Day 477 and Day 868), as well as when needed for surgery/procedures.

After BMN 270 infusion, the subject's ALT ranged from 9-48 U/L. His ALT exceeded 2x baseline starting at Week 9 (23 U/L), and was 28 U/L at Week 10 and 36 U/L at Week 11. He had 3 ALT assessments between 46-48 U/L from Day 85-107; no AE was assessed, and no corticosteroids were started for ALT elevation. Starting at Day 128 to the data cutoff, the subject's ALT levels ranged from 9-28 U/L. The subject's ALT level was 16 U/L at the last assessment before the data cutoff (Day 868).

On Day 109, therapeutic corticosteroids were started (prednisone 60 mg/day) for poor FVIII activity response (FVIII activity had risen as high as 45.1 IU/dL per chromogenic assay on Day 85, but had fallen to 10.5 IU/dL on Day 107). Despite starting corticosteroids, the subject's FVIII activity did not exceed 14.8 IU/dL for the remainder of the study prior to the data cutoff. The subject received corticosteroids from Day 109 to Day 276, with all doses between 5-10 mg after Day 193.

Subject

His first FVIII activity assessment after prophylaxis was 10.7 IU/dL (chromogenic) on Day 35. His FVIII activity peaked at Week 12 (65.1 IU/dL). At Weeks 49-52, the subject's median FVIII activity by chromogenic assay was 24.25 IU/dL.

As of the data cutoff, the subject had completed 115 weeks of post-infusion follow-up; his final FVIII assessment by chromogenic assay prior to the data cutoff was 7.8 IU/dL on Day 805.

The subject had an ABR for treated bleeds from Week 5 to the last visit of 0.9 bleeds/year. Starting at Week 5, he recorded 3 bleeding events.

The subject's screening ALT was 33 U/L. After BMN 270 infusion, his ALT remained within the normal range throughout the study period to the data cutoff, with the exception of a single timepoint (51 U/L, Day 595). This ALT elevation was not assessed as an adverse event, and the subject was not started on reactive corticosteroids or other immunosuppressants. At the next assessment, the ALT had returned to normal (35 U/L, Day 609). The subject's ALT level was 31 U/L at the last assessment before the data cutoff (Day 805).

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials	1/151	0/151	0/151
Non Controlled Trials	0/151	0/151	0/151

In the table above, the total number (n=151) includes all dosed subjects in 270-201 (n=15), 270-203 (n=1), 270-301 (n=134), and 270-302 (n=1).

2.6.5.4. In vitro biomarker test for patient selection for efficacy

To determine whether patients are AAV5 seronegative, the AAV5 total binding antibody (TAb) assay has been developed as a companion diagnostic in collaboration with ARUP Laboratories (ARUP AAV5 TAb CDx) and is intended for a CE-mark.

For Phase 3 studies, screening, confirmation, and titre cut points, sensitivity, and selectivity were assessed in haemophilia A plasma. AAV5 antibodies were measured in post-dose plasma samples from clinical trial patients and results were evaluated for association with consequences on patient efficacy and safety.
The assay technology is described and validated: it is a bridging immunoassay (plate coated with AAV5 capsid + patient's plasma + ruthenylated AAV5 capside to generate electrochemiluminescence (ECL) signal) that detect antibodies to AAV5 capsid. The LoD (limit of detection) was calculated to be 29 ng/mL total IgG.

The assay is intended to be used to determine patient eligibility for treatment with BMN 270, an AAV5-based gene therapy by excluding patient with pre-existing antibodies against adeno-associated virus serotype 5 from natural exposure.

The MAH has clarified that the assay is currently CE-marked (i.e., self-certified by the manufacturer) under Directive 98/79/EC on *in vitro* diagnostic medical devices (IVDD). The assessment of this IVD application by a Notified Body as per the new Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) is currently ongoing.

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

Comparison of results of Studies 270-201 and 270-301

Table 22 Baseline Disease Characteristics Across Studies

Attribute	270-201		270-301			
	4E13 vg/kg (N=6)	6E13 vg/kg (N=7)	Directly Enrolled (N=22)	270-902 Rollover (N=112)	mITT (N=132)	ITT (N=134)
Age at enrollment, years						
n	6	7	22	112	132	134
Mean (SD)	31.3 (9.6)	30.4 (5.8)	30.9 (8.7)	31.8 (10.6)	31.4 (10.1)	31.7 (10.3)
Min, Max	22, 45	23, 42	18, 52	19, 70	18, 70	18, 70
Race, n(%)						
Asian	0	1 (14.3)	2 (9.1)	17 (15.2)	19 (14.4)	19 (14.2)
Black or African American	1 (16.7)	0	1 (4.5)	14 (12.5)	15 (11.4)	15 (11.2)
Native Hawaiian or other Pacific Islander	0	0	0	1 (0.9)	1 (0.8)	1 (0.7)
White	5 (83.3)	6 (85.7)	18 (81.8)	78 (69.6)	94 (71.2)	96 (71.6)
Not provided due to patient privacy	0	0	1 (4.5)	2 (1.8)	3 (2.3)	3 (2.2)
Baseline type of FVIII treatment for hemophilia A, n(%)						
Prophylaxis	6 (100)	6 (85.7)	22 (100)	112 (100)	132 (100)	134 (100)
Episodic	0	1 (14.3)	0	0	0	0
Baseline ABR, treated bleeds/year						
n	6	7	22	112	132	134

Attribute	270-201		270-301			
	4E13 vg/kg (N=6)	6E13 vg/kg (N=7)	Directly Enrolled (N=22)	270-902 Rollover (N=112)	mITT (N=132)	ITT (N=134)
Mean (SD)	12.17 (15.35)	17.57 (14.71)	8.41 (19.90)	4.83 (6.47)	5.43 (10.04)	5.42 (9.96)
Median	8.00	24.00	0.93	2.80	2.04	2.30
Min, Max	0.0, 41.0	0.0, 40.0	0.0, 91.5	0.0, 33.1	0.0, 91.5	0.0, 91.5
Baseline ABR, n (%)						
0 bleeds/year	1 (16.7)	1 (14.3)	7 (31.8)	36 (32.1)	43 (32.6)	43 (32.1)
> 0 to 4	2 (33.3)	1 (14.3)	9 (40.9)	33 (29.5)	41 (31.1)	32 (31.3)
> 4 to 10	0	1 (14.3)	1 (4.5)	28 (25.0)	28 (21.2)	29 (21.6)
> 10	3 (50.0)	4 (57.1)	5 (22.7)	15 (13.4)	20 (15.2)	20 (14.9)
Baseline annualized FVIII usage, IU/kg/yr						
n	6	7	22	112	132	134
Mean (SD)	4704.16 (2345.75)	4444.48 (1969.50)	4889.11 (1477.54)	3961.17 (1751.47)	4111.30 (1747.82)	4113.52 (1738.96)
Median	4944.56	5085.90	4785.87	3754.42	3860.30	3860.30
Min, Max	1241.4, 7682.7	573.2, 6438.5	2550.9, 2885.0	1296.4, 11251.1	1296.4, 11251.1	1296.4, 11251.1
Baseline annualized FVIII infusions, infusions/year						
n	6	7	22	112	132	134
Mean (SD)	142.83 (48.78)	120.12 (45.94)	146.08 (78.91)	135.87 (51.99)	138.12 (57.22)	137.54 (57.04)
Median	155.83	121.38	119.26	128.56	125.09	121.12
Min, Max	53.8, 184.3	27.4, 158.5	49.3, 358.7	39.5, 363.8	39.5, 363.8	39.5, 363.8
History of previous diseases, n(%)						
Liver disease	1 (16.7)	0	2 (9.1)	7 (6.3)	8 (6.1)	9 (6.7)
Hepatitis B	1 (16.7)	0	3 (13.6)	17 (15.2)	18 (13.6)	20 (14.9)
Hepatitis C	2 (33.3)	2 (28.6)	8 (36.4)	33 (29.5)	39 (29.5)	41 (30.6)
HIV	0	0	2 (9.1)	0	0	2 (1.5)
Number of target joints, n(%)						
0	2 (33.3)	1 (14.3)	15 (68.2)	82 (73.2)	95 (72.0)	97 (72.4)
1	1 (16.7)	3 (42.9)	4 (18.2)	13 (11.6)	17 (12.9)	17 (12.7)
2	1 (16.7)	2 (28.6)	0	9 (8.0)	9 (6.8)	9 (6.7)
3	1 (16.7)	1 (14.3)	2 (9.1)	6 (5.4)	8 (6.1)	8 (6.0)
> 3	1 (16.7)	1 (14.3)	1 (4.5)	2 (1.8)	3 (2.3)	3 (2.2)

Subjects in 270-201 and 270-301 studies are similar, i.e. male with severe HA without FVIII inhibitors and without pre-existing immunity against AAV5.

A higher baseline median ABR in subjects in the Cohort 6E13 of the study 270-201 compared to the ITT population of study 270-301, i.e. 24.00/year (mean 17.57) and 2.30/ year (mean 5.42) respectively, and 57.1% of subjects in Cohort 6E13 Study 270-201 had a baseline ABR > 10 compared to 14.9% in Study 270-301 (ITT population). The target joint involvement at baseline is also higher in subjects from study 270-201 than study 270-301: only 1 of 7 subjects (14.3%) in the 6E13 vg/kg cohort had no target joint involvement, compared with 97/134 (72.4%) of subjects in the ITT Population of 270-301. Nevertheless the high-quality well-documented historical data collected in Study 270-301 appeared more reliable than the HA history collected in medical record in subjects of study 270-201 and might mitigate the variations observed among these two populations.

- *FVIII activity*

Overall, 100% of subjects (7/7) in the 6E13 vg/kg cohort of 270-201 and 88% of subjects (118/134) in the ITT Population of 270-301 achieved median FVIII activity levels at Week 49-52 in the range of mild haemophilia (5-40 IU/dL); this included 6/7 (86%) of subjects in the 6E13 vg/kg cohort of 270-201 and 50/134 subjects (38%) in 270-301 in the nonhaemophilic range (> 40 IU/dL).

Table 23 FVIII Activity (Chromogenic Assay) Levels at 6-Month Intervals in 270-201 and 270-301

Median FVIII Activity, IU/dL	270-301		270-201	
	ITT Population dosed > 2 years prior to data cutoff (N=19**)	ITT Population (N=134)	4E13 Cohort (N=6)	6E13 Cohort (N=7)
Weeks 23-26				
n	19	134	6	7 (1 at Week 28)
Mean (SD)	41.4 (40.6)	52.6 (54.8)	18.0 (8.7)	71.0 (41.6)
Median	32.7	38.1	18.0	61.2
Min, Max	0.0, 169.4	0.0, 367.3	3.9, 26.9	6.8, 121.6
Weeks 49-52				
n	19	134	6	7
Mean (SD)	39.3 (48.8)	42.5 (45.3)	21.1 (12.3)	63.6 (36.5)
Median	23.9	23.9	23.8	60.3
Min, Max	1.6, 207.4	0.0, 231.2	1.5, 36.5	12.5, 126.6
Week 76				
n	19 (1 imputed as 0)	38 (1 imputed as 0)	6	7 (all Week 80)
Mean (SD)	25.8 (29.6)	26.3 (29.0)	20.6 (15.4)	53.9 (31.2)
Median	14.8	14.1	21.3	50.2
Min, Max	0.0, 117.0	0.0, 117.0	0.0, 39.3	6.8, 106.5
Week 104				
n	19 (1 imputed as 0)	19 (1 imputed as 0)	6 (2 LOCF)	7 (1 LOCF)
Mean (SD)	22.4 (28.8)	22.4 (28.8)	12.3 (8.2)	36.4 (26.3)
Median	13.9	13.9	11.6	26.2
Min, Max	0.0, 110.6	0.0, 110.6	0.0, 22.4	3.9, 86.0
Week 156				
n			6	7
Mean (SD)			9.9 (9.0)	32.7 (32.8)
Median			7.9	19.9
Min, Max			0.0, 22.4	4.1, 100.1
Week 208				
n				7
Mean (SD)				21.3 (23.9)
Median				14.7
Min, Max				2.7, 71.2

LOCF, last observation carried forward

** 1 subject lost to follow-up after Week 66; subsequent FVIII activity levels imputed at 0 IU/dL

As previously noted in the withdrawn MAA, at Weeks 23-26 the median FVIII activity level in the Cohort 6E13 of 270-201 study was noticeably higher than those achieved by subjects from the interim analysis in 270-301 study as of latest DCO, but the range of values was wider in Study 270-301 than -201. This trend is confirmed with the updated data at Weeks 49-52 from the current application with a median FVIII activity at 23.9 and 60.3 IU/dL in subjects treated at 6E13 vg/kg in Study 270-301 and Study 270-201 respectively.

A decrease of FVIII activity levels over the time is also observed until Week 104 in study 270-301 and Week 208 in study 270-201. It is noted that at Week 208 in Cohort 6E13 Study 270-201 the min FVIII activity level was 2.7 IU/dL, meaning that all subjects treated (N=7) had a correction of severe haemophilia (<1 IU/dL) that persists at 4 years.

It is also supported by the comparison of $AUEC_{0\text{-}52W}$ showing that the median FVIII exposure is clearly higher in subjects from Cohort 6E13 vg/kg study 270-201 than subjects from study 270-301, i.e. 2480 and 1530, respectively. The difference in mean FVIII activity seems driven by the rate of subjects achieving a median FVIII activity level ≥ 40 IU/dL at Weeks 49-52, i.e. 85.7% in Cohort 6E13 of 270-201 study and 37.5% in Study 270-301.

Table 24 FVIII Activity (CS Assay) PD Parameters after 4E13 vg/kg and 6E13 vg/kg BMN 270 (Study 270-201)

Dose (vg/kg)		t_{max} (wk)	E_{max} (IU/dL)	AUEC 0-16w (IU*wk/dL)	AUEC 0-26w (IU*wk/dL)	AUEC 0-52w (IU*wk/dL)	AUEC 0-104w (IU*wk/dL)	AUEC 0-156w (IU*wk/dL)	AUEC 0-208w (IU*wk/dL)	E_{avg} 0-16w (IU/dL)	E_{avg} 0-26w (IU/dL)	E_{avg} 0-52w (IU/dL)	E_{avg} 0-104w (IU/dL)	E_{avg} 0-156w (IU/dL)	E_{avg} 0-208w (IU/dL)
4E13	N	6	6	6	6	6	6	6	0[a]	6	6	6	6	0	0[a]
	Mean	34.3	42.9	124	310	901	1850	2410	-	7.73	11.9	17.3	17.8	15.5	-
	SD	17.1	21.8	68.5	149	471	1140	1580	-	4.28	5.72	9.06	11.0	10.1	-
	Min	16.0	8.40	16.9	52.6	85.5	98.6	126	-	1.06	2.02	1.64	0.948	0.809	-
	Median	29.6	48.7	143	362	1020	2020	2450	-	8.96	13.9	19.5	19.4	15.7	-
	Max	64.4	68.9	194	476	1480	3360	4490	-	12.1	18.3	28.5	32.3	28.8	-
	CV%	49.8	50.7	55.4	47.9	52.3	61.8	65.4	-	55.4	47.9	52.3	61.8	65.4	-
6E13	N	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	Mean	34.2	117	321	1050	2880	5300	7240	8570	20.1	40.3	55.5	51.0	46.4	41.2
	SD	9.48	64.7	189	632	1530	2690	4160	5500	11.8	24.3	29.5	25.9	26.6	26.4
	Min	21.1	19.8	50.7	119	441	817	907	998	3.17	4.56	8.47	7.85	5.82	4.80
	Median	31.3	96.9	312	1020	2480	5670	7100	7870	19.5	39.1	47.7	54.5	45.5	37.8
	Max	46.1	215	624	2000	5270	8670	13700	18200	39.0	77.0	101	83.4	87.7	87.7
	CV%	27.7	55.5	58.8	60.4	53.2	50.7	57.4	64.1	58.8	60.4	53.2	50.7	57.4	64.1

t_{max}, time to maximum effect; E_{max}, maximum effect; AUEC, area under the effect curve; E_{avg}, mean effect over the interval. AUEC and E_{avg} parameters were only calculated for subjects with data available equal to or exceeding the upper time of the interval based upon actual relative time, except for $AUEC_{0\text{-}156w}$ and $E_{avg0\text{-}156w}$ in which a 4 week window was used, and $AUEC_{0\text{-}208w}$ and $E_{avg0\text{-}208w}$ in which a 12 week window was used.

[a] Subjects in the 4E13 vg/kg cohort had not reached the 208-week timepoint at the time of the datacut date for this analysis

Table 25 FVIII Activity (CS Assay) PD Parameters after 6E13 vg/kg BMN 270 (Study 270-301; ITT Population)

	t_{max} (week)	E_{max} (IU/dL)	$AUEC_{0\text{-}16W}$ (IU*week/dL)	$AUEC_{0\text{-}26W}$ (IU*week/dL)	$AUEC_{0\text{-}52W}$ (IU*week/dL)	$AUEC_{0\text{-}78W}$ (IU*week/dL)	$AUEC_{0\text{-}104W}$ (IU*week/dL)	$E_{avg0\text{-}16W}$ (IU/dL)	$E_{avg0\text{-}26W}$ (IU/dL)	$E_{avg0\text{-}52W}$ (IU/dL)	$E_{avg0\text{-}78W}$ (IU/dL)	$E_{avg0\text{-}104W}$ (IU/dL)
N	134	134	134	134	134	35	17	134	134	134	35	17
Mean	27.4	84.5	392	906	2140	3160	3630	24.5	34.9	41.2	40.5	34.9
SD	13.5	81.9	407	877	2100	2870	3490	25.4	33.7	40.3	36.8	33.5
Min	2.00	4.00	0.00	1.93	19.2	420	488	0.00	0.0742	0.369	5.38	4.69
Median	25.7	61.2	261	602	1530	2400	2470	16.3	23.2	29.3	30.8	23.7
Max	69.3	463	2500	4650	11300	12100	15200	156	179	217	155	146
CV%	49.1	96.9	104	96.8	97.7	90.8	96.1	104	96.8	97.7	90.8	96.1

t_{max}, time to maximum effect; E_{max}, maximum effect; AUEC, area under the effect curve; E_{avg}, mean effect over the internal. AUEC and E_{avg} parameters were only calculated for subjects with data available within a 2-week window prior to or exceeding the upper time of the interval based upon actual relative time.

A triphasic pattern of response was observed in 270-301 up to Year 2 follow-up and 270-201 up to Year 3 and Year 4 follow-up for the 4E13 vg/kg and 6E13 vg/kg cohorts, respectively.

This is characterised by a **rapid increase in FVIII activity** within approximately the first 6 months, followed by **an initial decline**, apparently proportionate to peak FVIII expression, followed by a **more gradual decline thereafter**, with FVIII activity sufficient to support hemostatic efficacy for multiple years.

The median (range) peak FVIII level was **96.9 (19.8, 215) IU/dL** for the 6E13 vg/kg cohort of 270-201, occurring at a median of **31.3 weeks**, and **61.2 (4.0, 463) IU/dL** for the ITT Population of 270-301, occurring at a median of **25.7 weeks.**

It is then expected that a higher peak of FVIII activity will translate into a higher FVIII activity at Year 2 or 3 and into a longer duration of clinically relevant FVIII activity levels. It is observed that the peak of FVIII activity occurred later in Study 270-201 than study 270-301 (31.3 weeks and 25.7 weeks respectively), leading to a later decline. Also the initial decline of the FVIII activity is more pronounced in study 270-301 compared to study Cohort 6E13 270-201 during the first year following the BMN 270 administration; the median FVIII activity even reached almost similar levels at Week 23-26 and Week 49-52 in Cohort 6E13 study 270-201, i.e. 61.2 and 60.3 IU/dL. From Week 76, a plateau is observed until Week 104 in study 270-301 (14.1 IU/dL at Week 76 and 13.9 IU/dL at Week 76) while a continuous decline over the time is shown in study 270-201 (50.2 IU/dL at Week 76, 26.2 IU/dL at Week 104, 19.9 IU/dL at Week 156 and 14.7 IU/dL at Week 208). This trend should however be interpreted with caution since only 38/134 (25.4%) of subjects in Study 270-301 reached Week 76 and 19/134 (14.2%) reached Week 104.

- *Annualised Bleeding Rate*

There was a clear decline of the mean ABR from baseline to Week 5 to DCO in subjects treated with 6E13 vg/kg from both Study 270-201 and Study 270-301 with a larger effect observed in subjects from Cohort 6E13 vg/kg Study 270-201 compared to Study 270-301 (95.8% and 84.2%, respectively) to be contextualised with the higher mean ABR at baseline in Cohort 6E13 Study 270-201 than in Study 270-301 (i.e. 17.57 and 5.42 bleeding episodes/year, respectively) and the longer mean follow-up in Cohort 6E13 Study 270-201 (1475.71 days, approximately 4 years) than in Study 270-301 (468.86 days, approximately 1.28 years). The 19 subjects in study 270-301 dosed ≥ 2 years before DCO reported a mean (SD) ABR from Week 5 to the data cutoff of 1.04 (2.01) (median 0.0) bleeding episodes/year representing a 88.3% decrease from baseline, which is in favour of a protective treatment effect over 2 years but the number of patients remains limited.

Table 26 Summary of ABR (Treated Bleeds) Requiring Exogenous FVIII Replacement Treatment

	Baseline	Post-Baseline				
		Weeks 1-4		Week 5 to Data Cutoff		
Cohort	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Follow-Up (Days)
270-201 4E13 vg/kg (N=6)						
Mean (SD)	12.17 (15.35)	1.17 (1.33)	13.32 (15.17)	2.67 (5.13)	0.88 (1.72)	1143.33 (50.03)
Median	8.00	1.00	11.41	0.50	0.17	1143.50
Min, Max	0.0, 41.0	0.0, 3.0	0.0, 34.2	0.0, 13.0	0.0, 4.4	1091, 1195
270-201 6E13 vg/kg (N=7)						
Mean (SD)	17.57 (14.71)	1.57 (1.99)	17.94 (22.69)	3.00 (7.51)	0.74 (1.86)	1475.71 (33.37)
Median	24.00	1.00	11.41	0.00	0.00	1475.00
Min, Max	0, 40	0, 6	0, 68.5	0.0, 20.0	0.0, 5.0	1427, 1539

Cohort	Baseline	Post-Baseline				
		Weeks 1-4		Week 5 to Data Cutoff		
	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Bleeding Episodes (no.)	ABR (no/yr)	Follow-Up (Days)
270-301 Population Dosed ≥ 2 Years Before Data Cutoff (N=19)						
Mean (SD)	8.91 (21.28)	0.32 (0.82)	3.60 (9.36)	2.47 (4.94)	1.04 (2.01)	805.58 (121.12)
Median	0.94	0.00	0.00	0.00	0.00	824.00
Min, Max	0.0, 91.5	0.0, 3.0	0.0, 34.2	0.0, 20.0	0.0, 7.9	431, 1021
270-301 Directly Enrolled Population (N=22)						
Mean (SD)	8.41 (19.90)	0.27 (0.77)	3.11 (8.76)	2.55 (4.65)	1.13 (1.93)	786.09 (122.98)
Median	0.93	0.00	0.00	0.00	0.00	820.00
Min, Max	0.0, 91.5	0.0, 3.0	0.0, 34.2	0.0, 20.0	0.0, 7.9	431, 1021
270-301 Rollover Population (N=112)						
Mean (SD)	4.83 (6.47)	0.15 (0.38)	1.73 (4.39)	0.83 (3.16)	0.78 (2.99)	406.54 (70.12)
Median	2.80	0.00	0.00	0.00	0.00	388.50
Min, Max	0.0, 33.1	0.0, 2.0	0.0, 22.8	0.0, 29.0	0.0, 27.3	326, 627
270-301 mITT Population (N=132)						
Mean (SD)	5.43 (10.04)	0.17 (0.47)	1.99 (5.37)	1.05 (3.46)	0.82 (2.85)	463.77 (158.27)
Median	2.04	0.00	0.00	0.00	0.00	389.00
Min, Max	0.0, 91.5	0.0, 3.0	0.0, 34.2	0.0, 29.0	0.0, 27.3	326, 1021
270-301 ITT Population (N=134)						
Mean (SD)	5.42 (9.96)	0.17 (0.47)	1.96 (5.33)	1.11 (3.49)	0.84 (2.84)	468.86 (162.50)
Median	2.30	0.00	0.00	0.00	0.00	389.50
Min, Max	0.0, 91.5	0.0, 3.0	0.0, 34.2	0.0, 29.0	0.0, 27.3	326, 1021

Max, maximum; Min, minimum; SD, standard deviation; ABR, annualized bleeding rate.

The annualized number of bleeding episodes, or annualized bleeding rate is defined as (Number of bleeding episodes during the calculation period / Total number of days during the calculation period) * 365.25.

Pre-infusion ABR was based historical data collected at Screening visit.

- *Exogenous FVIII use*

A large effect on exogenous FVIII use further to a single BMN 270 infusion is observed in subjects treated with the intended dosage 6E13 vg/kg in both studies 270-201 and 270-301 with a reduction from baseline to Week 5 to DCO of 98.2% in -301 and 96.3% in -201. The results in 270-301 population dosed ≥ 2 years before DCO suggest a long-term effect on exogenous FVIII use with a reduction of 99.3% from baseline to Week 5 to DCO.

Table 27 Summary of Annualised Exogenous FVIII Usage

Cohort	Baseline	Post-Baseline				
		Weeks 1-4		Week 5 to Data Cutoff		
	Annualized FVIII Usage (IU/kg/yr)	FVIII Usage (IU/kg)	Annualized FVIII Usage (IU/kg/yr)	FVIII Usage (IU/kg)	Annualized FVIII Usage (IU/kg/yr)	Follow-Up (Days)
270-201 4E13 vg/kg (N=6)						
Mean (SD)	4704.16 (2345.75)	125.48 (108.21)	1432.19 (1235.14)	663.91 (1473.33)	221.29 (493.70)	1143.33 (50.03)
Median	4944.56	114.54	1307.35	78.64	25.19	1143.50
Min, Max	1241.4, 7682.7	0, 304.8	0, 3478.8	0.0, 3670.1	0.0, 1228.7	1091, 1195
270-201 6E13 vg/kg (N=7)						
Mean (SD)	4444.48 (1969.50)	85.34 (76.03)	974.10 (867.87)	664.20 (1336.46)	163.10 (330.64)	1475.71 (33.37)
Median	5085.90	62.71	715.76	15.59	3.99	1475.00
Min, Max	573.2, 6438.5	0, 222.8	0, 2542.7	0.0, 3595.2	0.0, 890.3	1427, 1539
270-301 Population Dosed ≥ 2 Years Before Data Cutoff (N=19)						
Mean (SD)	4770.01 (1534.49)	301.48 (144.25)	3441.10 (1646.45)	348.72 (653.77)	148.52 (271.96)	776.79 (153.18)
Median	4635.03	286.74	3272.85	80.24	31.68	824.00
Min, Max	2550.9, 7885.0	40.9, 608.7	466.5, 6947.7	0.0, 2140.1	0.0, 892.2	373, 1021
270-301 Directly Enrolled Population (N=22)						
Mean (SD)	4889.11 (1477.54)	296.79 (138.75)	3387.53 (1583.69)	352.44 (609.86)	156.23 (255.69)	786.09 (122.98)
Median	4785.87	292.16	3334.69	83.06	42.08	820.00
Min, Max	2550.9, 7885.0	40.9, 608.7	466.5, 6947.7	0.0, 2140.1	0.0, 892.2	431, 1021
270-301 Rollover Population (N=112)						
Mean (SD)	3961.17 (1751.47)	268.50 (130.95)	3064.72 (1494.64)	63.07 (213.62)	56.89 (194.58)	406.54 (70.12)
Median	3754.42	241.62	2757.89	0.00	0.00	388.50
Min, Max	1296.4, 11251.1	43.1, 816.3	492.0, 9317.6	0.0, 1637.7	0.0, 1510.5	326, 627

Cohort	Baseline	Post-Baseline				
		Weeks 1-4		Week 5 to Data Cutoff		
	Annualized FVIII Usage (IU/kg/yr)	FVIII Usage (IU/kg)	Annualized FVIII Usage (IU/kg/yr)	FVIII Usage (IU/kg)	Annualized FVIII Usage (IU/kg/yr)	Follow-Up (Days)
270-301 mITT Population (N=132)						
Mean (SD)	4111.30 (1747.82)	271.89 (132.09)	3103.33 (1507.74)	96.18 (285.21)	67.26 (196.93)	464.19 (158.07)
Median	3860.30	249.53	2848.19	0.00	0.00	389.00
Min, Max	1296.4, 11251.1	40.9, 816.3	455.6, 9317.6	0.0, 2140.1	0.0, 1510.5	326, 1021
270-301 ITT Population (N=134)						
Mean (SD)	4113.52 (1738.96)	273.15 (132.14)	3117.72 (1508.26)	110.58 (329.22)	73.20 (208.05)	468.86 (162.50)
Median	3860.30	250.17	2855.50	0.00	0.00	389.50
Min, Max	1296.4, 11251.1	40.9, 816.3	466.5, 9317.6	0.0, 2140.1	0.0, 1510.5	326, 1021

Post-hoc variability analyses

There was a wide range of median FVIII activity levels achieved during Weeks 49-52 in mITT Population subjects, as measured by the chromogenic assay (0-231.2 IU/dL) and onestage assay (0-311.1 IU/dL).

Multiple post-hoc analyses were performed for each study population to assess potential trends between FVIII activity levels and baseline or demographic characteristics or postinfusion characteristics. Additionally, the potential trend between intrinsic and extrinsic factors and FVIII

activity pharmacodynamic parameters Emax, Emed (median FVIII activity between Week 49 and 52), and Eavg0-52w were evaluated for the 270-301 mITT Population and included the following characteristics: baseline age, race, body weight, body mass index (BMI), baseline ABR, baseline annualised FVIII usage, history of hepatitis B, history of hepatitis C, region, country, site, baseline ALT, FVIII genotype, von Willebrand factor levels, duration of study drug administration, concomitant medication use (including cumulative corticosteroid dose administration in first year and alternative immune suppressants), and baseline creatine phosphokinase.

Inter-individual variability does not appear to be associated with baseline age, bodyweight, body mass index (BMI), baseline annualised bleed rate, baseline annualised FVIII usage, history of Hepatitis B, history of Hepatitis C, region, country, site, baseline ALT, FVIII genotype, von Willebrand factor levels, duration of study drug administration, concomitant medication use (including cumulative corticosteroid dose administration in first year and alternative immune suppressants) and baseline creatine phosphokinase.

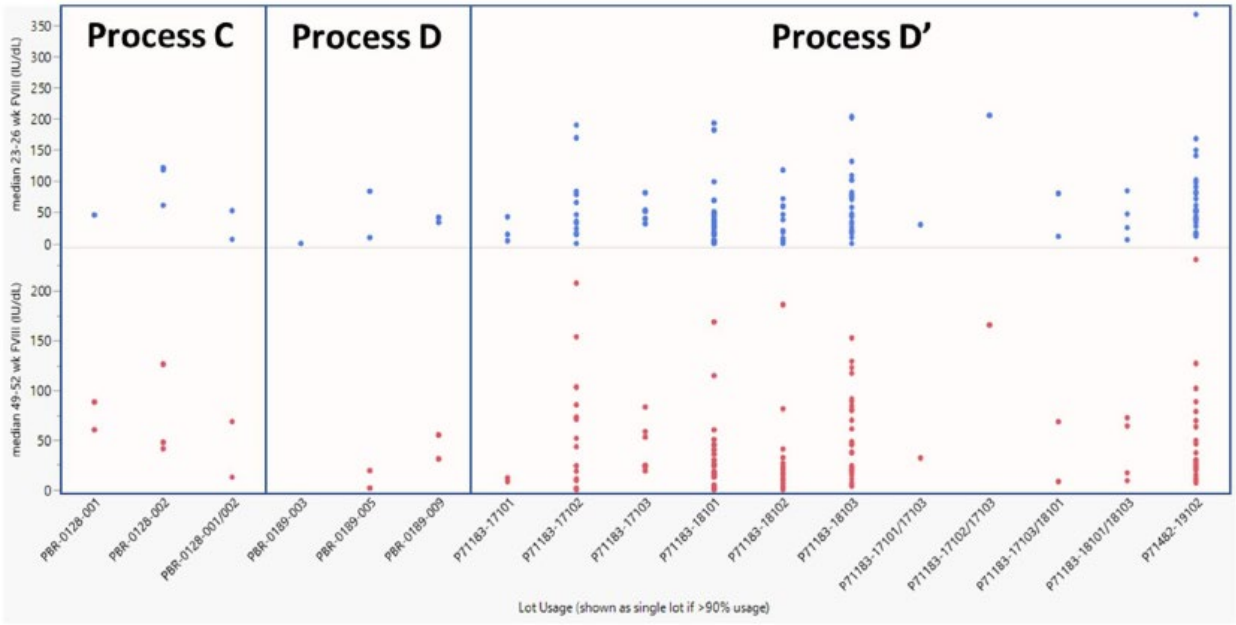
Although the majority of the inter-individual variability was left unexplained by this analysis, a trend of lower FVIII activity levels at week 49-52 was observed in **Black or African-American subjects** within the study population: median (range) of **10.5 (0, 83.3) IU/dL** in Black or African-American compared to **25.1 (0, 231.2) IU/dL in White** (n=96) and **42.4 (4.3, 166) IU/dL in Asian** (n=19) subjects. ABR and annualised FVIII utilisation data for Black or African-American subjects was consistent with that observed for other races. The number of subjects available for this analysis is small (N=15), and 10/15 of the subjects were treated at a single site, although site was investigated as a confounder and did not appear to explain the difference in response. The trend of decreased FVIII activity levels within BMN 270 in Black or African-American subjects is likely not to be confirmed since no consistency is demonstrated. Despite the applicant's explorations, there remains uncertainties regarding the inter-individual variability observed in FVIII activity levels. The responder/non-responder status is still unpredictable and a treatment failure cannot be foreseen at individual level.

Manufacturing Variability factors

No clear association was noted between FVIII activity and the BMN 270 drug product used to dose 270-201 or 270-301 subjects. BMN 270 used in the clinic was manufactured using Process C, D or D'. All material used to support the clinical studies met lot release specification. At the time of process change, material was subjected to a comprehensive physiochemical characterisation analysis to demonstrate suitable comparability. Process C material was used in 270-201 and Process D and D' material were used in 270-301.

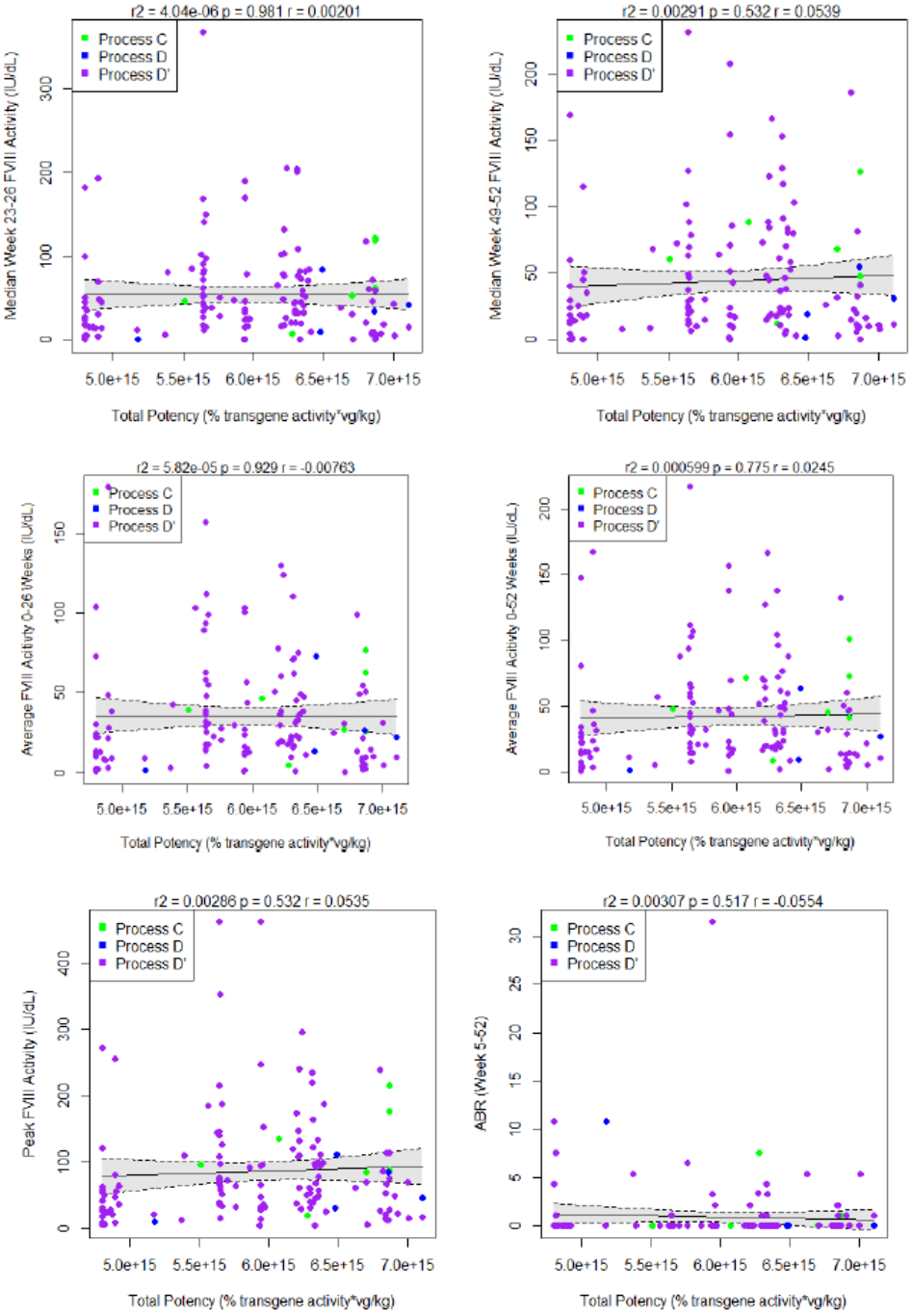
The graph below presents median Week 23-26 and median Week 49-52 FVIII activity levels for subjects dosed at 6E13 vg/kg from the 270-201 and 270-301 studies as a function of drug product lot.

Figure 11 Median Week 23-26 and Median Week 49-52 FVIII Activities



A wide range of Median Week 23-26 and Median Week 49-52 FVIII Activities is observed in all product lots from different manufacturing processes, which is more apparent in Process D' taking into account the higher number of subjects receiving a product lot from this process. The provided correlation plots of the investigation of relationship between drug product potency and clinical outcome including Median FVIII activity at Weeks 49-52 showed no meaningful correlations between any clinical outcome and drug product potency.

Figure 12 Correlation Plots: Clinical Outcome Versus Total Potency



Median FVIII activity between Weeks 23 and 26 and between Weeks 49 and 52 (top row), average FVIII activity between 0 and 26 weeks and between 0 and 52 weeks post-dose (estimated by the area under the FVIII activity-time curve between 0 and 26 weeks or 0 and 52 weeks divided by the time interval; middle row), peak FVIII activity (bottom left) and annualized bleeding rate between Weeks 5 and 52 (ABR; bottom right) by total potency for subjects dosed with 6E13 vg/kg BMN 270 from Process C (green), D (blue) or D′ (purple) in 270-201 or 270-301. Each datapoint represents an individual subject. Please note, multiple datapoints are overlapping at ABR=0 in the ABR by total potency figure.

Corticosteroid Dosing Recommendation

Data from 270-201 and 270-301 show that both early corticosteroid initiation prior to an increase in ALT and reactive use when encountering ALT elevation are effective approaches in aiding transgene

expression. While it may be hypothesised that early use of corticosteroids in the 270-201 6E13 vg/kg cohort may have mitigated the effects of hepatic inflammation due to AAV and contributed to the higher peak FVIII activity levels observed compared to 270-301, the disproportionate numbers of subjects who received early versus reactive corticosteroids in a non-randomised manner make it difficult to definitively determine the best corticosteroid regimen to achieve optimal long-term FVIII expression.

Thus, proposed labelling recommendations for temporary, adjunctive corticosteroid use after BMN 270 administration include the regimens used in both 270-201 and 270-301 to inform individualised prescriber-patient treatment decisions, as follows:

- Early Corticosteroid Regimen: Consider initiating an early oral corticosteroid regimen 2 weeks after administration of BMN 270 at a daily dose of 40 mg prednisone (or equivalent dose of another glucocorticoid) for 4 weeks. The corticosteroid dose can then be gradually tapered in a stepwise manner over 11 weeks.
- Patients with ALT increases above 1.5 x baseline or above ULN within the first 2 weeks post-BMN 270 administration or during corticosteroid tapering should follow the reactive corticosteroid regimen.
- Reactive Corticosteroid Regimen: If a patient's ALT rises above 1.5x baseline ALT or above ULN in the absence of an alternative cause, consider initiation of a reactive corticosteroid regimen at a daily dose of 60 mg prednisone (or equivalent dose of another glucocorticoid) for 2 weeks, which can be gradually tapered in a stepwise manner over 6 weeks. The tapering of corticosteroids for both regimens may be individualised based on the course of FVIII activity levels and hepatic function, taking into account the patient's medical condition, corticosteroid tolerance and the potential for withdrawal.
- Use of corticosteroids beyond 5 months after administration in patients who have not reached FVIII activity levels of at least 5 IU/dL is not recommended. There is limited benefit of initiating a new corticosteroid course to preserve or augment transgene expression in response to ALT elevation in these patients.
- There is limited information with regards to the benefit of starting a new corticosteroid course after the first year of BMN 270 administration.

Results from Study 270-301 suggested that duration of the corticosteroids use and the total corticosteroid dose between the two populations compared (Rollover and directly enrolled) likely not impact the FVIII activity. The labelling recommendations therefore provides limitation in the duration of the corticosteroid use which is supported.

Comparison of corticosteroid use based on the three main corticosteroid regimens used is summarised in Table 28. The data confirm earlier initiation of corticosteroid use in the 270-201 6E13 vg/kg cohort compared to the 270-301 populations, which was expected given protocol-mandated early use in 270-201. The reduction in ALT threshold to initiate corticosteroids in the Rollover Population compared to the Directly Enrolled Population did not substantially change the time from BMN 270 infusion to initiation of reactive corticosteroids; however, it did reduce the time from the first ALT elevation to the start of corticosteroids. In addition, the change in how corticosteroids were tapered in the Rollover Population (ie, corticosteroid tapering initiated only after ALT returned to baseline) resulted in approximately twice the total mean corticosteroid dose and a 35% increase in duration of corticosteroid use in the Rollover Population compared to the Directly Enrolled Population.

Table 28 Comparison of Corticosteroid Use in 270-201 and 270-301

	270-201	270-301	
	6E13 vg/kg (N=7)	Directly Enrolled (N=22)	Rollover (N=112)
Median FVIII activity* at Weeks 49-52, IU/dL	60.3	23.5	24.2
Median FVIII activity* at Weeks 23-26, IU/dL	61.2	33.2	38.6
Subjects with use of corticosteroids, n (%)	7 (100%)	14 (63.6%)	92 (82.1%)
Time from BMN 270 infusion to first use, weeks (Mean/Median)	3.0/3.1	9.9/8.2	11.0/7.9
Number of courses per subject (Mean/Median)	1.6/2.0	2.4/2.0	2.0/2.0
Total duration of courses per subject, days (Mean/Median)	149.7/162.0	179.1/156.0	241.9/244.0
Total dose per subject, mg (prednisone equivalents) (Mean/Median)	4088.6/4300.0	4658.7/4217.5	9260.4/6896.0
Time from BMN 270 infusion to first ALT ≥ 1.5x ULN or (ALT > ULN and > 2x baseline value), weeks (Mean/Median)	14.00/12.93	11.81/9.29	15.39/8.29

* Using chromogenic assay

Data from 270-201 and 270-301 suggest that both the early or reactive corticosteroid approaches can be effective for early support of transgene expression. The optimal timeframe of corticosteroid initiation has not been established, with limited data from 270-201 suggesting there may be a benefit to early initiation of corticosteroids to mitigate the effects of early-onset inflammation due to AAV potentially prior to, and associated with, initial ALT elevations and its potential impact on FVIII activity. Median FVIII activity at Weeks 49-52 was highest in the 270-201 6E13 vg/kg cohort (60.3 IU/dL), with similar, lower levels observed in both the Directly Enrolled and Rollover Populations (23.5 and 24.2 IU/dL, respectively). Of note, while the more prompt reactive initiation of corticosteroids to lower ALT increases may have led to the higher Median FVIII activity at Weeks 23-26 as seen in the Rollover Population compared with the Directly Enrolled Population, FVIII activity in the two groups was similar at 1 year. These data from 270-301 demonstrate that extending the dose and course of corticosteroids in the Rollover Population did not lead to substantial differences in median FVIII activity at Week 52 compared to the Directly Enrolled Population, suggesting a shorter dose of corticosteroids could be utilised without adversely impacting efficacy.

Evaluation of immune response after BMN 270 administration by IFN-γ ELISpot assay demonstrates that AAV5 capsid-specific cellular immune responses were moderately correlated with an increase in ALT levels, further supporting the potential benefit of corticosteroid use after BMN 270 administration. The early occurrence of these responses suggests that potentially proinflammatory events may be occurring prior to the advent of ALT elevations, of relevance when considering the proposed timing of early corticosteroid initiation. In the analysis performed for the AAV5 capsid-specific cellular immune responses and ALT elevation and FVIII activity, it was concluded that there was no significant association between the AAV5-specific cellular immune response and lower FVIII activity. Therefore, any impact of corticosteroids on FVIII activity through the AAV5 capsid-specific cellular immune response appears unlikely at this stage.

Quantitative quantitative pharmacokinetic model in order to extrapolate mean and median FVIII activity levels beyond the 2-year period.

A quantitative pharmacokinetic model was developed, using a linear mixed effects (LME) approach, to obtain population and individual estimates of FVIII activity half-life in order to extrapolate mean and median FVIII activity levels beyond the 2-year period.

Key Assumptions

The model assumed first-order elimination kinetics with random effects for subject on slope and intercept. Visual inspection of FVIII activity over time data, together with ANOVA tests for linearity and evaluation of model diagnostics with varying start times, supported Week 76 as the appropriate start of first-order elimination phase.

Data and Data Handling

The model was developed using FVIII activity data (CSA) from 270-301. All FVIII assessments collected within 72 hours of exogenous FVIII use or after resumption of prophylactic FVIII use were excluded from this analysis. Data from subjects who met one or more of the following exclusion criteria were also excluded during model development:

- Insufficient follow-up, defined as the last evaluable FVIII assessment < 100 weeks after administration of BMN 270 (5 subjects),
- ≥ 50% BLQ records in the lambda z region (i.e., between Week 76 and 104; 8 subjects),
- Resumption of prophylactic FVIII or starting emicizumab prior to end of lambda z region (i.e., Week 104; 4 subjects).

A total of 14 subjects were removed from model development (please note 3 subjects met more than 1 exclusion criteria). The final LME model dataset included 926 observations from 120 subjects.

Methods

Ln-transformed FVIII activity values from Week 76 to Week 104 were fit to the LME model with random effects for subject on slope and intercept using a restricted maximum likelihood (REML) method with the lmer package in R. The precision of parameter estimates and model diagnostics were evaluated to confirm goodness-of-fit.

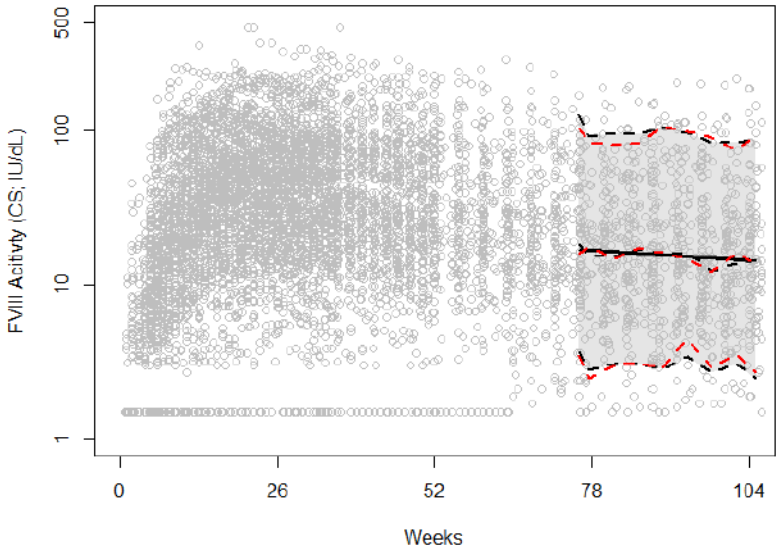
Results

The model estimated typical half-life (95% CI) calculated from the slope was estimated to be 123 (83.6, 232) weeks.

Table 20.14: Parameter estimates for the LME model

Fixed effects	Parameter	Typical Value	se (%)	Lower 2.5th	Upper 97.5th
Random effects	Intercept	3.25	4.97	2.94	3.57
	Slope	-5.65e-3	23.8	-8.29e-3	-2.99e-3
	Groups	Name	Variance	SD	Corr
	SUBJID	(Intercept)	2.068	1.44	
		Time (Weeks)	8.809e-5	9.39e-3	-0.70
	Residual		8.25e-2	0.287	

Figure 20.4: Visual Predictive Check Plot for the final LME model



The circles denote individual observed data, and the dashed red lines denote 5th, 50th, and 95th percentiles of the observed data; the solid black line represents population fit and the dashed black lines denote the median and 90% prediction intervals calculated from simulations (n=1000).

Extrapolation Beyond 2-year Period

Individual fits were used to extrapolate individual FVIII activities for Years 3, 4, and 5. Extrapolated values below the lower limit of quantification (LLOQ; 3 IU/dL) were set to 0 for descriptive statistics. Extrapolated FVIII activities were also set to 0 for subjects who were excluded from LME model due to having ≥ 50% of records <LLOQ in the lambda z region or due to having resumed prophylactic FVIII or started emicizumab prior to end of lambda z region (values imputed for 11 of the 14 subjects excluded from the LME model). Individual FVIII activities were extrapolated or imputed for a total of 131 subjects. The resulting mean and median FVIII activity values suggest long-term durability of response and hemostatic efficacy for the majority of subjects through Year 5.

Table 20.15: Extrapolated FVIII Activity (CSA) for 270-301 6E13 vg/kg subjects

Visit	FVIII Activity (CSA; IU/dL)		
	Mean (SD)	Median [min, max]	# (%) Subjects < 5 IU/dL
Week 104	**22.3** (29.7)	**11.1** [BLQ, 171]	**30 (22.9)**
Week 156	**16.9** (25.0)	**8.9** [BLQ, 156]	**40 (30.5)**
Week 208	**13.6** (22.4)	**7.2** [BLQ, 143]	**54 (41.2)**
Week 260	**11.8** (21.0)	**5.7** [BLQ, 131]	**63 (48.1)**

Additional Qualification of Approach

The model and extrapolation approach was further qualified by comparing the observed and predicted values for a subset of 270-301 subjects with available Week 156 FVIII activity data. The observed mean and median FVIII activity (17.1 and 9.3 IU/dL, respectively) at Year 3 were comparable to predicted values (16.7 and 12.4 IU/dL).

Additionally, the same modeling and extrapolation approach was applied independently to 7 subjects who received 6E13 vg/kg dose in 270-201 trial to compare the observed vs. predicted FVIII activity

values through Year 5. The results demonstrate good agreement between predicted and observed values. It is worth noting the predicted mean values were slightly less than the observed for all timepoints except Week 260, which may be due to the relatively small number of subjects included in the 270-201 model and resulting increased sensitivity of the mean to individual subject estimates.

Taken in context with the observed FVIII activity and reduction in ABR in 270-201 subjects with over 5 years of follow-up, BioMarin anticipates that FVIII activity and hemostatic efficacy will extend beyond 5 years for the majority of patients treated.

First assumption is that final linear PD decrease phase starts at week 76; the applicant mentions ANOVA testing which was not provided, and inspection from average plot below cannot ascertain the beginning of this final phase. This is insufficiently justified, and is the first weakness of the model.

In data handling, exclusion of subjects who had resumption of prophylactic FVIII before end of terminal phase may be introducing a bias for long-term-only sensitive patients in this modelling, and therefore overestimate the slope of the final decrease phase.

This overestimation may even be seen in comparisons between observed and predicted activity: as seen in Table 20.16 at week 260: mean activity predicted is 1.6 fold higher than mean activity observed, and the maximum median range goes from 35 to 55. Those two observations lead us to consider the model is overestimating activity at later times.

The applicant provided further justification regarding the assumptions behind the proposed approach. Based on the additional information, the selection of Week 76 as starting point for the final log-linear phase of activity was found acceptable. However, the applicant is reminded that it is expected that the model will be further updated using the additional data points that will be available to develop the updated model based on data from the pivotal study up to 5 years to enable predicting beyond 5 years.

2.6.5.6. Supportive study

Study 270-201: Liver biopsy substudy

A total of **5** liver biopsy samples have been obtained from participants treated across 3 dose levels in the 270-201 liver biopsy substudy, ranging **from 2.7 to 4.1 years post-treatment**, and with different FVIII activity profiles (including two dosed with 6E13 vg/kg BMN 270 and 2 participants in the 4E13 vg/kg cohort with notably different FVIII activity levels). Mild steatosis was observed in 4 out of 5 participants.

Table 29 Demographics of 270-201 Liver Biopsy Sub-Study Participants

Participant	BMN 270 Dose (vg/kg)	Age (at enrollment)	Biopsy at weeks (years)	ALT at time of biopsy (U/L)	FVIII Activity	
					CS (IU/dL)	OS (IU/dL)
[redacted]	6E12	25	W201 (Y3.86)	29	*	*
[redacted]	4E13	37	W140 (Y2.69)	11	18.6	28.4
[redacted]	4E13	37	W148 (Y2.85)	20	<3	2.1
[redacted]	6E13	32	W214 (Y4.12)	12	8.2	14
[redacted]	6E13	23	W213 (Y4.10)	11	13.5	23.9

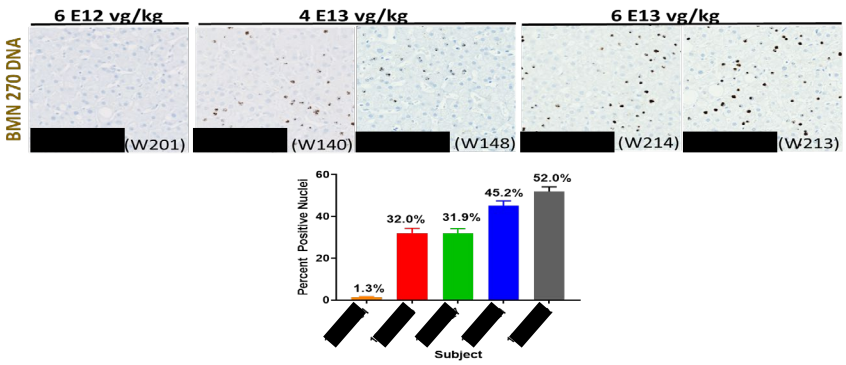
CS: chromogenic substrate assay; OS: one-stage assay

* Valid FVIII Activity results not available for 1546-1001 as this subject has been frequently using replacement FVIII on-demand post-BMN 270 infusion due to lack of appreciable FVIII expression at this low dose. CS, chromogenic FVIII activity. OS, one-stage FVIII activity.

Hepatocytes stained positive for BMN 270 genomes in a dose-dependent manner, which should be taken with caution due to the small number of liver biopsies:

- In the subject dosed with 6E12 vg/kg, 1.3% of hepatocytes stained positive for BMN 270 DNA.
- In the 2 4E13 vg/kg dosed subjects, 32.0 and 31.9% of hepatocytes stained positive for BMN 270 vector genomes, respectively;
- in the 2 6E13 vg/kg dosed subjects, 45.2 and 52.0% of hepatocytes stained positive for BMN 270 vector genomes.

Figure 13 Dose-Dependent Increase in Number of Hepatocytes Stained Positive for BMN 270 Vector Genome > 2.6 Years

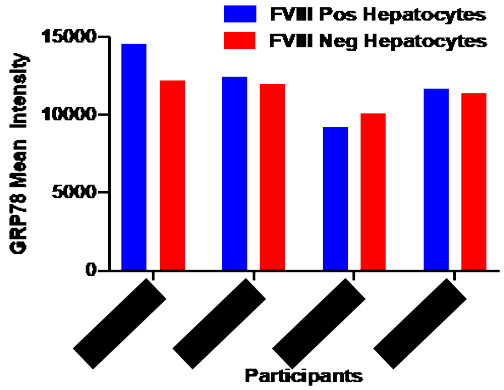


A lower responding subject had similar number of hepatocytes transduced with similar quantities and structures of BMN 270 episomes compared to a higher responding subject dosed at the same dose level (4E13 vg/kg). These data suggest that hepatocytes were successfully transduced in all subjects analyzed and the **mechanisms mediating the lower response in subject occurred in a post-transduction step.**

The percent hepatocytes staining positive for hFVIII protein in the endoplasmic reticulum (ER) is low, suggesting most hFVIII-SQ protein were secreted. For one subject (6E12 vg/kg, Week 201), 0.2% of hepatocytes stained positive for hFVIII-SQ protein in the ER. For two subjects (4E13 vg/kg, Week 140 and 4E13 vg/kg, Week 148), 1.63% and 1.03% of hepatocytes stained positive hFVIII-SQ protein in the ER, respectively. For two subjects (6E13 vg/kg, Week 214 and 6E13 vg/ kg, Week 213), 1.16% and 0.25% of hepatocytes staining positive for hFVIII-SQ protein, respectively.

hFVIII-SQ is a protein known to be inefficiently folded, thus concerns are raised that an adaptive unfolded protein response (UPR) leading to ER stress may be activated in instances of hFVIII-SQ protein expression in hepatocytes. Elevated Grp78 level is a biomarker of ER stress. In the human liver biopsies analyzed, the mean signal intensities of Grp78 staining in hepatocytes expressing hFVIII-SQ protein were not different from the mean signal intensities of Grp78 in hepatocytes not expressing hFVIII-SQ and there was no correlation between the signal intensities of hFVIII-SQ and Grp78 on a per cell basis.

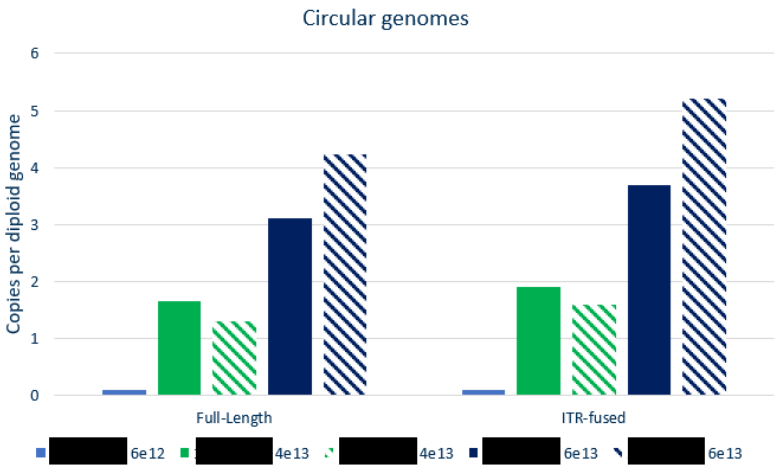
Figure 14 GRP78 Protein Intensity in Hepatocytes



Quantitative measurement of BMN 270 vector genome forms was performed by treating isolated liver DNA with various DNA digestion enzymes followed by ddPCR assays using different primers/probe sets. In all 5 participants, "R1-R11 linked full-length" episomes and ITR fusions were detected. The level of ITR fusions was similar to the level of full-length (R1-R11) genomes. Only slight increases in R2-R10 linked copies compared to R1-R11 linked copies were observed, suggesting ITR-D loop regions were mostly present (ie, not deleted) in the vector genomes 2-4 years following BMN 270 administration.

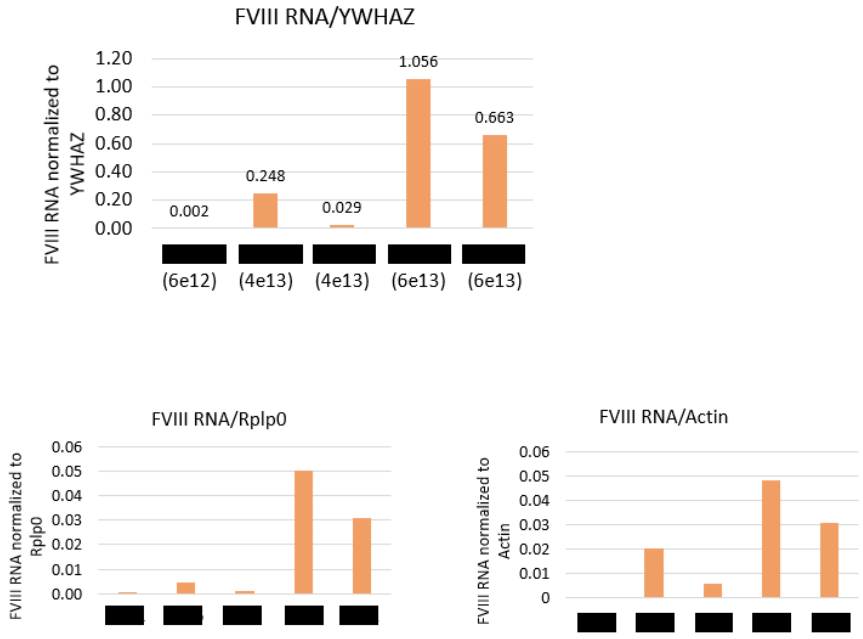
The amount of circular (PS-DNase resistant) full-length or ITR-fused BMN 270 vector genomes present in liver biopsies are dose-dependent (Figure 15). The levels of ITR fusions are similar to the levels of full-length genomes; suggesting majority of full-length genomes are ITR-fused.

Figure 15 Dose-Dependent Increase in Circular Full-Length and ITR-Fused BMN 270 Genomes Detected 2.6-4.1 Years Following Dosing



The provided data leads to the conclusion that the durability of presence of stable, circular episomes of the FVIII transgene in liver biopsies up to 4 years after BMN 270 treatment is likely not correlated to the FVIII activity. Indeed, for one subject treated at 4E13 vg/kg was FVIII non-responder (<3 IU/dL, CS) with a level of hFVIII-SQ RNA ~ 10-fold lower than the second subject treated at 4E13 vg/kg while the four other participants showed dose-dependent FVIII-SQ RNA levels. Data from one subject suggests that presence of circular BMN 270 vector genome in hepatocytes is likely not predictable of long-term FVIII-SQ RNA and plasma FVIII activity.

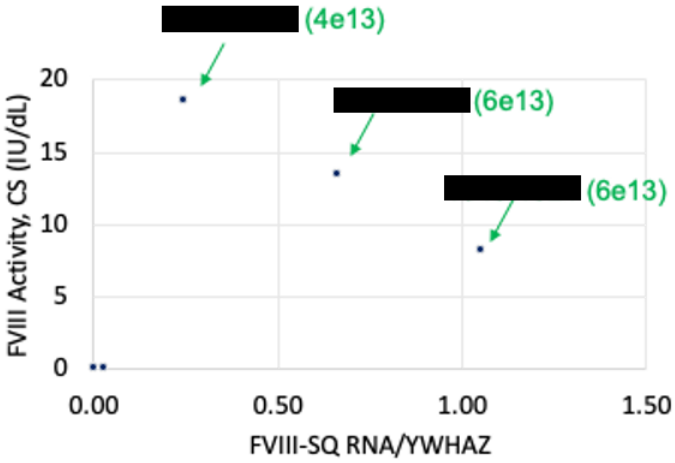
Figure 16 Levels of hFVIII-SQ RNA in Liver Biopsies



The low-responder subject had a similar number of hepatocytes transduced with similar quantities and structures of BMN 270 episomes as a more robust responding subject who received the same dose (4E13 vg/kg). These data suggest that hepatocytes were successfully transduced in all subjects analyzed and the mechanisms mediating the low response occurred a post-transduction step.

The levels of liver hFVIII-SQ RNA and plasma FVIII activity from the participants that had FVIII activity above quantitation limit were plotted. Higher hFVIII-SQ RNA levels did not result in higher circulating FVIII activities (Figure 17). This suggests that FVIII activity is likely not hFVIII-SQ RNA level-dependent.

Figure 17 Liver hFVIII-SQ RNA Levels Negatively Correlate with Levels of Plasma FVIII Activity



The mechanism(s) underlying the gradual decline in FVIII levels in the years following initial transduction has **not been elucidated thus far**. The Sponsor will continue to evaluate potential

mechanisms in translational and clinical studies. Investigations on mechanisms contributing to inter-subject variability in hFVIII-SQ RNA levels and on factors associated with level of hFVIII-SQ protein activity in plasma were performed. The results suggested multiple factors of variability such as the involvement of zinc in the increase of AAV transduction, copper metabolism in the increase or reduce of AAV transduction, possible alterations in the acetylation status of transcription factors due to a low expression of HDAC9 or natural variability of GRP78 in variability of FVIII activity detected in circulatory compartment. Overall the findings of the performed investigations need to be confirmed by additional data and, if possible, translated into clinical recommendation.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The efficacy data came from the dose-response study 270-201 and the pivotal study 270-301. In the current application, data from study 270-301 were based on 134 patients (ITT population) who have reached Week 49-52 and supportive efficacy data until Week 208 (4 years) were gained from 7 patients of the 15 enrolled in Study 270-201 that were treated at the intended dosage (6E13 vg/kg). Updated data were submitted in responses to CAT questions, i.e. 2-year data from Study 270-301 at DCO of 15 November 2021 (updated CSR not available) and 5-year data from Study 270-201 Cohort 6E13 vg/kg at DCO of 29 March 2021.

Study 270-201 was a first-in-human, phase I/II, dose escalation study with sequential enrolment. Based on the exploratory dose-finding study 270-201, the 6E13 vg/kg dose has been chosen for the confirmatory trial. As such, only 1 patient was included in cohort 1 (6E12 vg/kg) and 1 in cohort 2 (2E13 vg/kg) as the FVIII activity did not reach the target level of 5 IU/dl at W3 post-infusion. In Cohort 3 (6E13 vg/kg) a total of 7 subjects were included and 6 subjects were included in the additional cohort (Cohort 4) at a dose of 4E13 vg/kg. Liver biopsies were performed on 5 subjects and analyzed to better understanding of gene expression related to circularised genomes.

Study 270-301 was a Phase 3 non-randomised single arm open-label study and considered as the pivotal study. Included subjects are male ≥ 18 years of age with severe haemophilia A (residual FVIII levels ≤ 1 IU/dL), without history of FVIII inhibitor and without detectable pre-existing antibodies to the AAV5 capsid. Most of the enrolled subjects in the pivotal study 270-301 were coming from the non-interventional study 270-902 (N=112, Rollover population) while 22 subjects were directly enrolled. Baseline data of the rollover population was collected in study 270-902 whereas those from directly enrolled patients relied on historical data.

The prophylactic treatment authorised for subjects to be included is FVIII replacement therapy for at least 12 months. Emicizumab was added as prohibited medications starting 30 days before Screening and through the end of the study from Amendment 1 (25 January 2018) with the rationale that experimental haemophilia treatments are prohibited during the study as they could affect the assessment of FVIII levels in 270-301 subjects. It is a new therapeutic option indicated as prophylactic treatment since 2019 in a similar population, i.e. severe haemophilia A without a history of factor VIII inhibitors, requiring less treatment administrations than FVIII replacement therapy.

Reactive oral corticosteroids were primarily initiated if ALT ≥ 1.5xULN, and later amended to initiate reactive corticosteroids if ALT ≥ 1.5x ULN or ALT > ULN and >2x the subject's baseline ALT. A more conservative approach to manage the risk of elevation ALT was adopted further to the interim analysis within the Protocol Amendment 6 which lowered the threshold for initiation of corticosteroids if ALT > ULN or ALT 1.5x BL.

The efficacy study objectives and endpoints were similar to those from the withdrawn MAA but the timepoints for evaluation differed i.e. Weeks 49-52 instead of Weeks 23-26 in the withdrawn

application. Primary endpoint was the change of FVIII activity by chromogenic assay during Weeks 49-52 post-BMN 270 infusion from baseline; FVIII activity has been recognised as a marker of efficacy with established correlation with clinical endpoints. The approach of defining the primary endpoint on the surrogate, FVIII activity level, has been endorsed notably by the CHMP in a scientific advice for Roctavian (previously Roxavvy).

An optional liver biopsy was added in Protocol amendment 6, 3 April 2020 to gain knowledge in the BMN 270 transduction in hepatocytes and establish a correlation with the treatment response, but there was no data available at the submission date.

Several important changes were made to the study design and statistical methods of the pivotal trial compared to the original protocol as part of several protocol amendments and SAP versions, with impacts on the sample size, IAs, multiplicity adjustment procedure and primary / secondary analysis definitions. Due to the content and timing of these updates, it is not possible to consider the study type I error as being formally controlled. As a consequence, the applicant was requested to remove claims of statistical significance / superiority and associated p-values from the SmPC.

In this situation, the clinical relevance as well as the robustness of the primary and secondary endpoint analyses are key to the benefit assessment. Several supplementary analyses based on original protocol definitions were requested to assess their consistency with the analyses based on the final SAP.

The roll-over process for subjects in Study 270-902 to participate in Study 270-301 was not fully comprehensible from the submitted documents. On request the applicant provided additional tables and flow-charts describing the roll-over process, which clarified the outstanding issues. For the primary endpoint, the baseline value was imputed as 1 IU/dL for all subjects. This imputation was not specified in the original protocol and does not reflect the initial intent of a true change from baseline analysis. More generally, the applicant was requested to refrain from using the term "change of hFVIII activity by chromogenic assay during Weeks 49-52 post-BMN 270 infusion from baseline" when in fact "median hFVIII activity by chromogenic assay during Weeks 49-52 post-BMN 270 infusion (minus one)" is referred to. The corresponding text under SPC section 5.1 was adapted accordingly.

The LOCF algorithm for the primary endpoint was not specified in the original protocol, and it is not considered to be a conservative approach to missing data, especially when efficacy is anticipated to deteriorate over time. The applicant confirm that only 2 subjects had an imputed assessment for the primary endpoint and performed a supplementary analysis where missing post-treatment values are imputed with 0 instead. The results were consistent.

The descriptive statistics of the primary endpoint indicate that the distribution is positively skewed. Therefore, the one-sample t-test, which assumes data normality, may not be the most appropriate statistic. The applicant provided sensitivity analyses using a non-parametric test (Wilcoxon), as well as based on log-transformed levels. These analyses did not change the interpretation of the primary analysis results.

Efficacy data and additional analyses

Study 270-201

As of the data cutoff of 8 April 2020, all 15 subjects completed the post-infusion Week 156 study visit and 9 subjects (60%) (all except those in the 4E13 vg/kg cohort) have completed the post-infusion Week 208 study visit. The FVIII activity in Cohort 6E13 vg/kg (N = 7) up to Week 208 remained sustained, i.e. median FVIII activity level as mesured by CS assay of 16.4 (2.7-71.2) IU/dL at Week 208. The lower FVIII levels obtained in Cohort 4E13 vg/kg (N = 6) suggested a dose-response to BMN 270. The FVIII activity in Cohort 4E13 even lower than those in Cohort 6E13 remained clinically meaningful (median FVIII activity level of 7.9 IU/dL at Year 3, i.e. corresponding to mild HA). It is

however observed an increase of subjects with low FVIII activity levels is however observed over the time consistently with the observed FVIII decline. Three of 6 subjects in the 4E13 vg/kg cohort were low responders at Week 156: 1545-1025, 1546-1006 and 1545-1027 with a median FVIII activity by CSA of 3.4 IU/dL, 3.9 IU/dL and 0 respectively Two of the 7 subjects in the 6E13 vg/kg cohort were low-responders, i.e. 1583-1082 with a median FVIII activity by CSA of 3.1 IU/dL at Week 204 and 1545-1021 with a median FVIII activity of 0 at Week 208.

Updated data at 5 years were submitted in response to the List of Questions as of the data cut of 29 March 2021: 5 out 7 subjects reached FVIII activity levels > 5 IU/dL at Week 260 (year 5). The mean (SD) FVIII activity for the 6E13 vg/kg cohort was 11.6 (12.2) (median 8.2) IU/dL by CSA, supporting a long-term FVIII activity at haemostatic level. There was a 96% reduction in mean ABR over 260 weeks post-infusion in the 6E13 vg/kg cohort and the reduction of the mean AFU in the Rollover Population was by 99.9%, showing the FVIII activity levels achieved translation to a clinical relevance, notwithstanding that all subjects in the 4E13 vg/kg and 6E13 vg/kg cohorts remained off FVIII prophylaxis as of the data cutoff of the updated CSR. The low-responders reported higher ABR and exogenous FVIII use from Week 5 to beyond than the other responders; these two subjects reached a FVIII activity level <5 IU/dL earlier than subjects who had a longer hemostatic FVIII activity. The FVIII levels increases achieved by the treated-subjects were correlated with the observed reduction of ABR and FVIII utilisation, supporting the clinical relevance of BMN 270.

All the participants in the liver biopsies substudy showed BMN 270 dose-dependent hepatocyte transduction (1.3% of hepatocytes stained positive for BMN 270 vector genome at 6E12 vg/kg dose, ~ 32% at 4E13 vg/kg dose and between 45.2% and 52% at 6E13 vg/kg dose), but the limited number (N=5) limited any interpretation. The results showed that durability of presence of stable, circular episomes of the FVIII transgene in liver biopsies up to 4 years after BMN 270 treatment was likely not correlated to the FVIII activity according to data from one Subject treated at 4E13 vg/kg with hepatocytes successfully transduced and was FVIII non-responder (<3 IU/dL, CS) with a level of hFVIII-SQ RNA ~ 10-fold lower than the second subject treated at 4E13 vg/kg. Moreover results from 3 participants that had FVIII activity above the quantitation limit showed that higher hFVIII-SQ RNA levels did not result in higher circulating FVIII activities, suggesting that FVIII activity is likely not hFVIII-SQ RNA level-dependent and that the mechanisms mediating the low response in some subjects occurred in post-transduction steps. The results of the ongoing investigation on variability of FVIII expression beyond liver transduction suggested multiple factors of variability such as the involvement of zinc in the increase of AAV transduction, copper metabolism in the increase or reduce of AAV transduction, possible alterations in the acetylation status of transcription factors due to a low expression of HDAC9 or natural variability of GRP78 in variability of FVIII activity detected in circulatory compartment. To better understand the mechanisms involved in variability of FVIII expression and gradual FVIII levels decline to estimate the durability of sustained FVIII activity, the applicant committed to provide the results of optional liver biopsy substudies once available. These results will be submitted post-authorisation.

Study 270-301

An additional 19-months follow-up (DCO 16 November 2020) was initially submitted for Study 270-301 compared to the interim CSR submitted in the withdrawn application. In addition, the applicant submitted comprehensive data for all primary and secondary endpoints at two years (Week 104) for all subjects in the Phase 3 270-301 pivotal trial, including a subset of subjects who have at least three years of data. Of the 134 dosed subjects, 130 (97.0%) had completed 52 weeks of follow-up on study as of the data cutoff.

As of the data cut of 16 November 2020 at Weeks 49-52 post-infusion, the median FVIII activity level (chromogenic assay) was 23.92 IU/dL and the median [min, max] time to peak FVIII activity using the

chromogenic assay was 25.7 [2.00, 69.3] weeks in ITT population. The FVIII activity levels at Weeks 49-52 post-infusion are similar to FVIII level of mild haemophilia (5-<40 IU/dL) and are encouraging for a long-term use. Nevertheless beyond the median peak FVIII reached at approximately 6 months, FVIII activity levels decreased over the time, i.e. median and mean (SD) FVIII activity level (chromogenic assay) reached at Week 104 (N = 19) for the ITT Population were 13.9 UI/dL and 22.4 (28.8) UI/dL respectively. The durability of the effect remains unpredictable. It is still unknown if the FVIII activity decrease might be sufficient to support hemostatic efficacy for multiple years or decline below the threshold of 5 IU/dL or return to baseline value. Moreover the initial FVIII activity level decline is expected to vary among the treated patients providing that it is apparently proportionate to peak FVIII expression. Only a very long-term follow-up will help to elucidate this point; 5-year data will be provided in the framework of the Conditional marketing authorisation.

The applicant provided the primary analysis on ITT population (N=134) at Week 104 to further characterize the treatment effect, with inferential statistical analyses of long-term FVIII activity levels. The 2-year data in Study 270-301 (DCO 15 November 2021) are supportive of a sustained FVIII activity translating into clinical relevance with a reduction in ABR and annualised FU levels including the majority of patients that achieved FVIII activity levels <5 IU/dL. A decline of FVIII activity levels is however observed over time as expected. The number of subjects achieving FVIII activity levels <5 IU/dL increased between Year 1 and Year 2 from 16 to 33 out 134, and also the subjects that returned to continuous FVIII prophylaxis were 2 at Year 1 and 6 at Year 2. A quantitative pharmacokinetic model has been developed in order to extrapolate mean and median FVIII activity levels beyond the 2-year period.

There was a wide range of FVIII activity during Week 49-52 reflecting a high variability of FVIII levels among the treated-subjects, i.e. from 0 to 231.2 UI/dL in ITT population as measured by the chromogenic assay. Extra high levels of FVIII activity (>150 IU/dL) were reported in 7/134 (5.2%) patients at Weeks 49-52. A total of 15/134 (11.2%) subjects had FVIII activity level > 250 IU/dl at one or more timepoints. None of these subjects report any thromboembolic adverse events and subjects with history of arterial or venous thromboembolic events were excluded. The updated data showed that no thromboembolic adverse events was reported as of the DCO of 15 November 2021.

A total of 16/134 subjects (11.9%) had a median FVIII activity level <5 IU/dL at Weeks 49-52. For the large majority of these subjects (15/16, 93.8%), the FVIII level peak achieved remained lower than the median peak FVIII activity using the chromogenic assay of 61.2 IU/dL. Two subjects restarted a continuous prophylaxis after one year post-BMN 270 administration due to low FVIII levels. Despite their low median FVIII activity level at Weeks 49-52, 62.5% (10/16) of subjects with FVIII activity level < 5 IU/dL at Week 52 gained an improved ABR for treated bleeds from Week 5 to last visit. Two of the 16 subjects (12.5%) remained at ABR 0, the impact of BMN 270 remaining uncertain. Also significant reductions in the use of exogenous FVIII is observed in the 16 subjects having a median FVIII activity level <5 IU/dL at Weeks 49-52; BMN 270 reduced annualised FVIII utilisation by ≥90% in 75% (12/16) of these subjects. Clinically relevant effects can be observed in subjects with a median FVIII activity level <5 IU/dL with a mean follow-up period of 66.2 weeks (approximately 1 year and 3 months) but remain unpredictable. Additional data up to 104 weeks (2 years) were submitted. There was approximately 2 times more subjects with a FVIII activity level <5 IU/dL from year 1 to year 2, i.e. 16/134 (11.9%) and 33/134 (24.6%) respectively. Similarly to data at week 49-52, subjects with FVIII activity <5 IU/dL at week 104 gained an improvement in ABR with 14 out 33 (42.4%) having an ABR of zero, 8 (24.2%) having an ABR < 1 and 11 (33.3%) having an ABR > 1 of which three subjects with ABR >1 had a higher ABR post BMN 270 infusion compared to baseline (subjects 1591-3907, 1633-3005 and 1682-3902).

The secondary endpoints as of the DCO date of 15 November 2021 demonstrated relevant clinical benefit of the sustained FVIII levels with a clear decrease of FVIII use observed in the Rollover

population (N=112) with a decline of 98.2% of the annualised FVIII utilisation from baseline to Week 5 to last visit and a significantly reduction of ABR for treated bleeds observed in the Rollover population, i.e. a decline of 84.5% from mean baseline ABR to a mean ABR from Week 5 to the last visit as of the data cutoff. The majority of subjects in the Rollover population (74.1%) reported no treated bleeds from Week 5 to the last visit and a total of 11 subjects had more than 3 bleeds of which one Subject that experienced the maximum value of number of bleeding episodes (29) and ABR (27.3 /year) from Week 5 to data cutoff and was a non-responder with median FVIII activity <3.0 IU/dL at Week 52.

Overall the quality of life was improved from baseline to Week 52 based on the different QoL endpoints (i.e. HAEMO-QoL-A, EQ-5D-5L, Haemophilia Activities List, WPAI+CIQ:HS and PROBE. It should be emphasised that baseline scores were considered high despite the severe haemophilia which may be reflective of the 'disability paradox', i.e. patients that have been shown to adapt to their levels of disability, often reporting better quality QoL than expected from the general population. At Week 104, there were clinically meaningful improvements in HRQoL (Quinn 2020), as assessed by the Haemo-QoL-A Total score (improvements at Weeks 52 [6.34 (11.99), p<.0001] and 104 [6.92 (12.54), p<.0001] in 270-301) in the ITT population.

Comparison of Studies 270-201 (Cohort 6E13 vg/kg) and 270-301

The comparison of results of studies 270-201 Cohort 6E13 vg/kg and 270-301 showed that subjects from both studies were similar, i.e. male with severe HA without FVIII inhibitors and without pre-existing immunity against AAV5. However there was a higher baseline median ABR and target joint involvement in subjects in the Cohort 6E13 of the study 270-201 (N=7) compared to the study 270-301 (ITT population, N=134) suggesting a better controlled disease in subjects in Study 270-301 than those from Study 270-201. However the difference in the collect of historical data might mitigate the variations observed among these two populations, i.e. high-quality well-documented historical data collected in Study 270-301 appeared more reliable than medical record in subjects of study 270-201.

The median FVIII activity level at Week 49-52 was noticeably higher in the Cohort 6E13 of 270-201 study than in 270-301 study, i.e. median FVIII activity of 60.3 IU/dL and 23.9 IU/dL respectively. The difference in mean FVIII activity seems driven by the rate of subjects achieving a median FVIII activity level ≥ 40 IU/dL at Weeks 49-52, i.e. 85.7% in Cohort 6E13 of 270-201 study and 37.5% in Study 270-301. A decrease of FVIII activity levels over the time is also observed until Week 104 in study 270-301 and Week 208 in study 270-201. It is noted that at Week 208 in Cohort 6E13 Study 270-201 the min FVIII activity level was 2.7 IU/dL, meaning that all subjects treated (N=7) had a correction of severe haemophilia (<1 IU/dL) that persists at 4 years.

From the applicant, the FVIII activity follows a triphasic pattern of response, i.e. an initial peak within approximately the first 6 months, initial decline apparently proportionate to peak FVIII expression then a more gradual decline. It is then expected that a higher peak of FVIII activity will translate into a higher FVIII activity at Year 2 or 3 and into a longer duration of clinically relevant FVIII activity levels.

There was a clear decline of the mean ABR from baseline to Week 5 to the latest data cutoff in both subjects with a larger effect observed in subjects from Cohort 6E13 vg/kg Study 270-201 compared to Study 270-301 (96% and 84.5%, respectively) to be contextualised with the higher mean ABR at baseline in Cohort 6E13 Study 270-201 than in Study 270-301 (i.e. 17.57 and 5.42 bleeding episodes/year, respectively) and the longer mean follow-up in Cohort 6E13 Study 270-201 (approximately 5 years) than in Study 270-301 (approximately 2 years).

A large effect on exogenous FVIII use further to a single BMN 270 infusion is observed in subjects treated with the intended dosage 6E13 vg/kg in both studies 270-201 and 270-301 at the latest DCO with a reduction of 98.1% over the efficacy evaluation period in -301 and 96% over 260 weeks post-infusion in -201.

There was a wide range of median FVIII activity levels achieved during Weeks 49-52 in BMN 270 treated subjects. Post hoc FVIII activity levels variability analyses were performed by the applicant based on several baseline or demographic characteristics or postinfusion characteristics but the inter-individual variability was left unexplained. The observed trend of decreased FVIII activity levels within BMN 270 in Black or African-American subjects was likely not consistent. The responder/non-responder status is still unpredictable and a treatment failure cannot be foreseen at individual level. An update on the applicant's investigations on the identification of factors of FVIII activity levels variation was provided. Identified factors of variability such as race and FVIII genotype (intron 22 inversion status) remained inconclusive trends based on small subgroup numbers, lack of consistency with the analyses performed at one year and the nature of the analyses (post-hoc exploratory analyses) with limited reliability. When considering that non responders patients could not only experience the burden of (a potentially extended period of time with) corticosteroids and might be exposed to a risk of malignancy due to vector integration, it is important that the applicant pursue the investigations for predictive factors of non-response through ongoing studies (mainly 303, as well as 203 and 205). This is part of the specific obligation.

The impact of the use of corticosteroids was evaluated between the Directly enrolled (N=22) and the Rollover populations (N=112) in Study 270-301. Since the Directly enrolled population was enrolled in the study earlier than the Rollover population, the corticosteroids use was initiated at a higher ALT threshold for the majority of Directly enrolled population compared to the Rollover population. Despite a longer mean duration of corticosteroids use and an almost two times higher total corticosteroids dose in the Rollover population compared to the Directly enrolled population, the median FVIII activity was similar between these 2 populations. It is noted that the time from BMN 270 infusion to first corticosteroid use was similar across the 2 populations (mean (SD) time of 9.9 (4.2) and 11.0 (10.4) weeks in Directly Enrolled and Rollover population, respectively).

While comparing the corticosteroid use between Study 270-201 Cohort 6E13 and Study 270-301, the parameter leading to a variation of FVIII activity seems to be the timing of initiation of corticosteroid but the limited data collected in Study 270-201 Cohort 6E13 and different corticosteroids regimens in studies prevent any clear conclusion. The best timing for the initiation of the corticosteroids remains unknown.

While temporary corticosteroid regimens have been used in these and other clinical trials with AAV vectors to ameliorate liver toxicity and possibly rescue transgene expression, further study is required to more precisely determine the optimal corticosteroid regimen (eg, prophylactic immune suppression) and investigate other immunosuppressant drugs. In this regard, ongoing Study 270-303 endeavors to evaluate the efficacy and safety of BMN 270 with prophylactic corticosteroids in HA patients.

The initially proposed labelling on the corticosteroids use recommended both early corticosteroid initiation prior to ALT elevation and reactive use after ALT elevation; it also included that limited data suggest that initiating corticosteroids prior to ALT elevation may be beneficial and that optimal timing of initiation of corticosteroids has not been established. The absence of a clear conclusion on the most appropriate timing for the initiation of the corticosteroids precludes any statement on a suggested best approach.

Based on the current data, it was decided to recommend a reactive use of CS. In the future, the applicant should provide a more comprehensive CS use approach, once data becomes available from the ongoing Study 270-303 that intends to evaluate the efficacy and safety of BMN 270 at 6E13 vg/kg dose with prophylactic corticosteroids in HA patients (together with additional data being collected through studies 203 and 205 in adults patients with severe haemophilia A in some non-currently covered subgroups of patients, i.e. with FVIII inhibitors and anti-AAV5). These data will have to be submitted in the context of the CMA with the aim of establishing a CS regimen.

2.6.7. Conclusions on the clinical efficacy

Updated data at Week 104 submitted in Study 270-301 in 134 subjects showed that 75.4% of treated subjects achieved a sustained FVIII activity level ⩾5 IU/dL translated by an improvement of ABR and exogenous FVIII use. Nevertheless uncertainties on the clinical efficacy remain. The durability of the FVIII activity at sustained levels remain unknown as FVIII decline is observed after a peak reached at approximately 6 months. A total of 24.6% of subjects had a median FVIII activity level <5 IU/dL at Weeks 104. Clinically relevant effects can be observed in subjects with a median FVIII activity level <5 IU/dL with a mean follow-up period of approximately 2years but this remains unpredictable. The 5-year follow up data from the pivotal study will have to be provided as specific obligation in the context of the CMA.

The inter subject-variability in FVIII levels is still not elucidated and updated analyses with data derived from ongoing studies will be submitted as post-authorisation measures.

The impact of the corticosteroids use on the difference observed in FVIII activity levels between Study 270-201 Cohort 6E13 vg/kg and Study 270-301 is not confirmed and the best regimen of the corticosteroids remains unknown; the applicant will have to determine the adequate corticosteroid regimen through ongoing studies as a second specific obligation in the context of the CMA.

The liver biopsies showed that durability of presence of stable, circular episomes of the FVIII transgene in liver biopsies up to 4 years after BMN 270 treatment was likely not correlated to the FVIII activity. This suggests that mechanisms mediating the low response in some subjects occurred in post-transduction steps; the results of the optional liver biopsy substudies and the updated variability analyses should be submitted post-authorisation.

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

- In order to further characterise the long term efficacy and safety of Roctavian in adults with severe haemophilia A (congenital factor VIII deficiency) without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5), the MAH should conduct and submit the final results of:
    - study 270-401, a follow-up study of patients enrolled in the clinical studies.
    - study 270-801, a Retrospective Cohort Study of patients treated with Valoctocogene roxaparvocec based on data from a registry, according to an agreed protocol.

- In order to further characterise the long-term efficacy and to further inform on the risk-benefit balance of Valoctocogene roxaparvocec in adults with severe haemophilia A (congenital factor VIII deficiency) in a broader population, the MAH should conduct and submit the final results of the study 270-601.

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

- In order to confirm the efficacy and safety of Roctavian in adults with severe haemophilia A (congenital factor VIII deficiency) without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5), the MAH should submit the final results including 5 years follow-up of the phase 3, single arm study 270-301.

- In order to confirm the efficacy and safety of Roctavian, adequate corticosteroid regimen and to identify predictive factors for no or low response in adults with severe haemophilia A

(congenital factor VIII deficiency), the MAH should submit the final results of the phase 3 single arm study 270-303 in patients receiving a prophylactic corticosteroid regimen. Interim data from open- label studies 270-203 and 270-205 should also be provided.

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

2.6.8. Clinical safety

The Clinical Safety is based on safety results from 4 clinical studies of all investigated doses in 151 subjects with severe haemophilia A (HA) exposed to BMN 270 for up to 4.5 years, at the time of the resubmission. In responses to the CAT List of Questions, the applicant updated the data with the 2-years follow-up of all the patients included in 270-301 study and with the other following updated studies.

The 4 ongoing studies are:

- 270-201 (data cutoff 29 March 2021) – a Phase 1/2, Dose-Escalation, Safety, Tolerability and Efficacy Study of BMN 270 in Patients with Severe HA

- 270-203 (data cutoff 31 May 2021) – a Phase 1/2, Safety, Tolerability and Efficacy Study of 6E13 vg/kg BMN 270 in Patients with Severe HA who have pre-existing AAV5 antibodies

- 270-301 (data cutoff 15 November 2021) – the pivotal Phase 3 Open-Label, Single Arm Study to Evaluate the Safety and Efficacy of 6E13 vg/kg BMN 270 in Patients with Severe HA

- 270-302 (data cutoff 31 May 2021) – a Phase 3 Open-Label, Single Arm Study to Evaluate the Safety and Efficacy of 4E13 vg/kg BMN 270 in Patients with Severe HA.

2.6.8.1. Patient exposure

For the integrated safety analyses, 4 analysis populations were performed by the applicant:

- All Treated Population (n=151) is defined as all subjects from any of the 4 clinical studies included in this marketing application who received any dose of BMN 270.

- Proposed Label Population (n=141) is defined as any subject who received BMN 270 at the dose of 6E13 vg/kg, and who was also AAV5 TAb-negative.

- 6E13/AAV5-/HIV- Population (n=139) is defined as any subject who received BMN 270 at the dose of 6E13 vg/kg, and who was also HIV-negative and AAV5 TAb-negative.

- 4E13/AAV5-/HIV- Population (n=6) is defined as any subject who received BMN 270 at the dose of 4E13 vg/kg and who was also HIV-negative and AAV5 TAb-negative.

Cumulatively, 151 subjects have been dosed with BMN 270 in 1 of the 4 treatment studies. One subject in 270-301 withdrew at Week 66, and one subject died at Week 95; all other subjects (149/151, 98.7%) have completed the Week 104 post-infusion follow-up, while 33/151 (21.9%) had follow-up for more than 3 years, 15/151 (10.0%) for more than 4 years, and 8/151 (5.3%) for more than 5 years as of the individual study data cutoff dates. The total exposure as of the data cutoffs for all studies was 391.8 patient-years, including 352.8 patient-years at the 6E13 vg/kg proposed label dose.

Table 30 *BMN 270 exposure – updated*

	270-201 (N=15)	270-203 (N=1)	270-301 (N=134)	270-302 (N=1)
Total Follow-Up Time, person-years	**71.7**	**3.1**	**314.0**	**3.0**
Total weight-adjusted dose infused, E13 vg/kg				
n	15	1	134	1
Mean (SD)	4.74 (1.726)	6.5 (NA)	6.08 (0.38)	4.2 (NA)
Median	4.57	6.5	6.15	4.2
Min, Max	0.57, 6.6	6.5, 6.5	5.5, 7.2	4.2, 4.2
Duration of post-infusion follow-up, weeks				
n	15	1	134	1
Mean (SD)	249.45 (27.25)	159.6 (NA)	122.28 (24.06)	157.9 (NA)
Median	264.14	159.6	110.86	157.9
Min, Max	211.1, 287.1	159.6, 159.6	66.1, 197.4	157.9, 157.9
Duration of post-infusion follow-up, n (%)[b]				
≥ 52 to < 104 weeks	0	0	12 (9.0)[a]	0
≥ 104 to < 156 weeks	0	0	106 (79.1)[a]	0
≥ 3 years	0	1 (100)	16 (11.9)[a]	1 (100)
≥ 4 years	7 (46.7)[a]	0	0	0
≥ 5 years	8 (53.3)[a]	0	0	0

*dose-escalation study with dose levels: 6E12 vg/kg (1 subject), 2E13 vg/kg (1 subject), 4E13 vg/kg (6 subjects), or 6E13 vg/kg (7 subjects).

Regarding the proposed target population, subjects in the Proposed Label Population had a mean (SD) age of 31.6 (10.1) years, with a range of 18-70 years. This was similar to the All-Treated Population. Approximately 50% of subjects in each population were under 30 years of age, and approximately 10% were aged 50 or older, including only one patient aged 70 years. A particular vigilance is required on this population in its long-term follow-up.

2.6.8.2. Adverse events

All subjects experienced at least 1 treatment-emergent adverse event (TEAE) while on study.

Twenty-nine subjects (19.2%) in the All-Treated Population, and 25 subjects (17.7%) in the Proposed Label Population, experienced at least 1 SAE.

Thirty-two subjects (21.2%), including 24/134 subjects in 270-301 (17.9%), have experienced at least 1 Serious Adverse Event (SAE). Overall, at the time of resubmission's MA, across both populations the majority of the TEAEs (97%) were assessed as mild (Grade 1) or moderate (Grade 2) in severity based on the Common Terminology Criteria for Adverse Events (CTCAE) severity criteria. A majority of subjects (approximately 75% in both populations) experienced only Grade 1 or Grade 2 TEAEs; Grade 3 events occurred in 39 subjects (25.8%) in the All-Treated Population and 36 subjects (25.5%) in the Proposed Label Population. Two subjects (one each in 270-201 and 270-301) have reported Grade 4 events, all assessed as unrelated to BMN 270.

Importantly, all participants completed their full-dose infusion of BMN 270; no infusions required permanent termination prior to completion due to TEAEs. No subjects have discontinued study treatment during BMN 270 infusion or withdrawn from a study as a result of an AE.

One subject in 270-301 died of suicide,. The subject did not receive corticosteroids or other immunosuppressants at any time during 270-301 and had a long history of severe and often poorly controlled depression; the Investigator assessed the death as being unrelated to BMN 270.

The most commonly reported EOSI were increases in aminotransferases and infusion-related reactions. At the time of resubmission's MA, increases in aminotransferases included TEAEs of ALT elevation reported as an EOSI (129 subjects [85.4%] in the All-Treated Population, and 122 subjects [86.5%] in the Proposed Label Population). Infusion-related reactions were reported in 59 subjects (39.1%) in the All-Treated Population, and 53 subjects (37.6%) in the Proposed Label Population. In response to D120 LoQ, in 270-301, ALT elevation was reported as an EOSI in 119 subjects (88.8%). No thromboembolic events have been reported, and no subjects in any BMN 270 study have developed anti-FVIII neutralizing antibodies.

Table 31 Overall Summary of Adverse Events – updated

	270-201 (N=15)	270-203 (N=1)	270-301 (N=134)	270-302 (N=1)
Subject with any AE, n(%)[a]	**15 (100)**	**1 (100)**	**134 (100)**	**1 (100)**
AEs leading to dose adjustment during infusion	0	0	0	0
AEs leading to dose interruption during infusion	0	0	4 (3.0)	0
AEs leading to study drug discontinuation	0	0	0	0
AEs leading to study withdrawal	0	0	0	0
Subjects with any SAE, n(%)[a]	**7 (46.7)**	**1 (100)**	**24 (17.9)**	**0**
SAEs leading to dose interruption during infusion	0	0	3 (2.2)	0
Subjects with any treatment-related AE, n(%)[a]	**13 (86.7)**	**1 (100)**	**123 (91.8)**	**1 (100)**
Treatment-related SAEs	1 (6.7)	0	5 (3.7)	0
Subjects with any AE of CTCAE Grade ≥ 3, n(%)[a]	4 (26.7)	0	42 (31.3)	1 (100)
Subjects who died, n(%)[a]	0	0	1 (0.7)	0
Subjects with any EOSI, n(%)[a]				
ALT elevation reported as an EOSI	13 (86.7)	0	119 (88.8)	1 (100)
AEs of Liver Dysfunction	14 (93.3)	0	119 (88.8)	1 (100)
Potential Hy's law case	0	0	0	0
Infusion-associated events	8 (53.3)	1 (100)	50 (37.3)	0
Systemic hypersensitivity	0	1 (100)	7 (52.2)	0
Anaphylactic or anaphylactoid reactions	0	0	3 (2.2)	0
Thromboembolic events	0	0	0	0
Development of anti-FVIII neutralizing antibodies	0	0	0	0

AAV, adeno-associated virus; AE, adverse event; EOSI, event of special interest; SAE, serious adverse event; SMQ, Standardised MedDRA Query.
AEs with onset or worsening after initiation of the study drug through the study exit or data cutoff were included. Relationship to study drug was assessed by the investigator.
Infusion-associated events occurred with onset during the BMN 270 infusion or within 48 hours after the end of the infusion.

[a] Percentages were calculated using the total number of subjects (N) in each analysis population as the denominator. Subjects with more than one AE of the same category were counted only once for that category.

Adverse Events by Post-Infusion Time Interval (Proposed Label Population)

In the first 26 weeks after the infusion, 100% of subjects reported at least one TEAE, irrespective of causal relationship to BMN 270 infusion. The incidence of treatment-related TEAEs was higher during the first 26 weeks after the infusion (87.9%, 124 of 141 subjects) and dropped considerably after 26 weeks post-infusion (36.9%, 52/141 subjects during Weeks 27-52, and 7-12% beyond that).

Approximately half (30/65) of all AEs of Grade 3 or higher occurred during the first 26 weeks, and no Grade 3 events have been reported beyond Week 78.

It is noted that infusion-associated events occurred within the first 4 weeks after infusion. Hepatic reactions occurred most commonly between Weeks 4-26 and decline steadily thereafter. In response to D120 LoQ, infusion-associated events were finally reported in 50 (37.3%) subjects in 270-301.

Adverse Events by Severity

Of the 2521 TEAEs that have been reported across all studies and all populations in the BMN 270 clinical development programme, 2446 (97.0%) have been reported as Grade 1 or Grade 2 in severity. Sixty-seven (2.7%) Grade 3 TEAEs have been reported. Two subjects reported 5 total Grade 4 AEs (0.2%) (3 events had missing severity at the data cutoff).

Sixty-two of the 67 Grade 3 events occurred in subjects in the *Proposed Label Population*. The most commonly reported Grade 3 events have been ALT increased (11 subjects [7.8%]), AST increased (5 subjects [3.5%]), hypertension (3 subjects [2.1%]), CPK increased (2 subjects [1.4%]), diarrhoea (2 subjects [1.4%]), gastroenteritis (2 subjects [1.4%]), and weight increased (2 subjects [1.4%]) Eight subjects (5.2%) experienced 12 Grade 3 events that were assessed as related to concomitant immunosuppressant use, including diabetes (2 events in 1 subject), hypophosphatemia (2 events in 1 subject), hypertension (2 events in 2 subjects), and single events of influenza, pneumonia, CMV pneumonia, acne, weight gain, and rectal haemorrhage.

Only 5 Grade 4 events in 2 patients (each in clinical trials 270-201 and 270-301) and 1 Grade 5 event were reported, all assessed as unrelated to BMN 270. One patient (with elevated uric acid level at screening) treated in the 6E12 vg/kg cohort in clinical trial 270-201 experienced 2 serious grade 4 events: post-procedural hepatic haemorrhage (following a liver biopsy) and hyperuricemia (evaluated as a result of renal under excretion). One patient in clinical trial 270-301 experienced 3 serious Grade 4 depression AEs, all related to history of severe depression. During the late-breaking period after the data cut-off for this MA, this patient died of suicide.

In response to D120 LoQ, the applicant specified that All Grade 3 and 4 events except 1 (weight gain) had resolved.

Common Adverse Events

In the Proposed Label Population, the most commonly reported TEAEs have been ALT increased (122 subjects [86.5%]), headache (55 subjects [39.0%]), AST increased (50 subjects [35.5%]), nausea (52 subjects [36.9%]), and arthralgia (44 subjects [31.2%]). A similar pattern of TEAEs was seen in the All-Treated Population.

In response to D120 LoQ, the applicant specified that across both 270-201 and 270-301, the common AEs were generally consistent with the incidence rates in 270-201, influenced by longer exposure in the study. In 270-301, the most commonly reported TEAEs have been ALT increased (119 subjects [88.8%]), headache (55 subjects [41.0%]), arthralgia (53 subjects [39.6%]), nausea (51 subjects [38.1%]), and AST increased (47 subjects [35.1%]). The exposure-adjusted adverse event rate of TEAEs in 270-301 was 8.2 TEAEs/person-year.

Table 32 Adverse Events occurring in at least 10% of subjects – updated

Preferred Term, n(%)[a]	270-201 (N=15)	270-203 (N=1)	270-301 (N=134)	270-302 (N=1)
ALT increased	13 (86.7)	0	119 (88.8)	1 (100)
Headache	8 (53.3)	0	55 (41.0)	0
Arthralgia	11 (73.3)	1 (100)	53 (39.6)	0
Nausea	3 (20.0)	0	51 (38.1)	0
AST increased	9 (60.0)	0	47 (35.1)	0
Fatigue	6 (40.0)	0	40 (29.9)	0
Acne	3 (20.0)	0	36 (26.9)	0
Upper respiratory tract infection	6 (40.0)	0	33 (24.6)	0
Pyrexia	3 (20.0)	0	31 (23.1)	1 (100)
Nasopharyngitis	10 (66.7)	0	29 (21.6)	1 (100)
Diarrhoea	6 (40.0)	0	28 (20.9)	0
Insomnia	5 (33.3)	0	27 (20.1)	0
Back pain	6 (40.0)	0	25 (18.7)	0
Cough	6 (40.0)	0	24 (17.9)	0
Oropharyngeal pain	4 (26.7)	0	24 (17.9)	1 (100)
Weight increased	2 (13.3)	0	22 (16.4)	0
Vomiting	3 (20.0)	0	21 (15.7)	0
CPK increased	2 (13.3)	0	17 (12.7)	0
Myalgia	2 (13.3)	0	17 (12.7)	1 (100)
Cushingoid	1 (6.7)	0	16 (11.9)	0
Hypertension	1 (6.7)	0	16 (11.9)	0
Pain in extremity	5 (33.3)	0	16 (11.9)	0

[a] Percentages were calculated using the total number of subjects (N) in each analysis population as the denominator. Subjects with more than one AE of the same category were counted only once for that category.

Events of special interest (EOSI)

Abnormal Liver Tests (LTs)

o Alanine aminotransferase (ALT) elevation reported as EOSIs (Preferred term [PT]: "Alanine aminotransferase increased", reported as EOSI)

In the Proposed Label Population, elevations in ALT meeting the EOSI definition were observed in 122 of 141 subjects (59.0%). Of the 122 subjects, the highest CTCAE grade of ALT elevation was Grade 1 in 90 subjects (73.8%), Grade 2 in 21 subjects (17.2%), and Grade 3 in 11 subjects (9.0%). A total of 326 ALT EOSI were reported in 122 subjects; 274 of the 326 events (84.0%) were reported as Grade 1 in severity. The majority of events of ALT elevations were resolved as of the data cut-off. Several are ongoing. No subjects experienced a Grade 4 or Grade 5 ALT elevation, and no subjects met the Hy's law or drug-induced liver injury (DILI) criteria.

In response to D120 LoQ, the applicant specified that as of the new data cutoff, one additional Grade 3 ALT elevation has been reported in 270-301, in a subject with a previous Grade 3 ALT elevation and 68 new ALT EOSI (1 in 270-201, 67 in 270-301). In 270-301, 372 events (96.6%) were Grade 1 or Grade 2 in severity. 374 of 385 ALT elevation EOSI had resolved (97.1%). In the events which are not resolved as of the data cut, 2 events resolving and 9 remain unresolved. All these events are Grade 1 and only one subject remains on a tapering dose of immunosuppressant therapy. In response to D150, the applicant specified that all ALT elevation events ongoing at the MAA data cut-off were subsequently resolved and immunosuppressant treatment were discontinued.

As discussed above, the EOSI definition evolved during BMN 270 clinical trials (ALT ≥ 1.5x baseline, ALT ≥ 1.5x ULN, ALT > ULN and ALT > 2x baseline and finally ALT > ULN or ALT ≥ 1.5x patient's baseline value). Moreover, the recommendations on the use of corticosteroids to handle immune mediated hepatotoxicity have evolved over time in BMN 270 clinical trials.

Regarding the duration of events, the applicant stated that ALT elevations responded rapidly to corticosteroid treatment with no symptoms or sequelea suggestive of clinically significant hepatocellular injury or liver dysfunction. However, it is noted that the events were considered resolved more than 30 days after the AE onset in many patients up to 488 days despite the use of corticosteroids, and some patients had another events despite the ongoing corticosteroid treatment.

Of the 122 subjects in the Proposed Label Population who had TEAEs of ALT increased, 113 (92.6%) received oral corticosteroids (prednisone or prednisolone) per instructions in the protocol. A majority of subjects (at least 87 patients from 270-301) had discontinued corticosteroids at the time of the data cut-off. After Week 52, only one subject was initiated with a new course of corticosteroids for a Grade 3 ALT elevation.

Liver dysfunction, defined using the Medical Dictionary for Regulatory Activities (MedDRA) search strategy high level term (HLT = "Liver function analyses")

In the Proposed Label Population, events of liver dysfunction included 122 subjects with ALT elevation, along with 50 subjects with AST elevation, 5 subjects with GGT elevation, 2 subjects with elevated blood bilirubin, and 1 subject with elevated conjugated bilirubin. Of the non-ALT events of liver dysfunction, five subjects (all in 270-301) experienced non-serious Grade 3 AST elevations. All non-ALT elevation events were reported as non-serious, and in most cases events of elevated AST or bilirubin occurred concurrently with events of elevated ALT.

In most cases, the AST elevations accompanied ALT elevations. However, the opposite is not necessary true as it appears that in the Proposed Label Population, 122 patients had ALT elevations but only 50 had AST elevations. The applicant provided a full discussion based on literature review (non clinical and clinical data) on immune mediated hepatotoxicity associated with AAV vectors and clinical data with BMN 270 which are fully acknowledged. The mechanism of action of this toxicity is still not fully understood and a management of patients is well scheduled.

Potential Hy's law cases: ALT or aspartate aminotransferase (AST) ≥ 3x ULN and serum TBL > 2x ULN (assessments of ALT/AST and TBL must be on the same day)

It is acknowledged that no Hy's law cases (ALT or AST ≥ 3x ULN and serum TBL > 2x ULN) have been observed in all patients treated with BMN 270.

Finally, hepatotoxicity is considered as an important identified risks with some requirements concerning this risk including in the SmPC or in additional mitigation measures in Healthcare Professional Guide and Patient Card.

Corticosteroid-Related Adverse Events / AIS

Different approaches to corticosteroid use were employed in BMN 270 clinical trials. Management of corticosteroid treatment at 60 mg by day of prednisolone if the LFTs were abnormal was revised into using a prophylactic corticosteroid treatment (40 mg per day of prednisolone starting at week 3) to finally remove the requirement for prophylactic oral corticosteroids due to AEs observed. At the time of the data cut-off of the resubmission, the mean (SD) time from infusion to the first use of corticosteroids was 10.4 (9.7) weeks (median 7.7 weeks; range 1-66 weeks) in the Proposed Label Population. The mean (SD) time from infusion to the first use of corticosteroids was 3.0 (0.7) weeks (median 3.1 weeks; range 2-4 weeks) in the 6E13 vg/kg cohort of 270-201 and 10.9 (9.8) weeks (median 8.1 weeks; range 1-66 weeks) in the ITT population of 270-301; within 270-301, the mean (SD) time from infusion to first use of corticosteroids 9.9 (4.2) weeks in the Directly Enrolled Population (median 8.2 weeks; range 4-20 weeks) and 11.0 (10.4) weeks in the Rollover Population (median 7.9 weeks; range 1-66 weeks).

The mean (SD) duration of corticosteroid use per subject in the *Proposed Label Population* was 32.7 (16.4) weeks (median 32.4 weeks; range 3-79 weeks). However some patients treated with 6E13 vg/mL BMN 270 had been treated for at least 6 months with corticosteroids. Dependence of corticosteroids could be disputable for these patients.

Regarding the total dose of corticosteroids administered, subjects in 270-301 (mean 8668.7/median 6560.0 mg) received on average more than twice the total dose of corticosteroids compared with subjects in the 270-201 6E13 vg/kg cohort (mean 4088.6 mg/median 4300 mg) mainly due to subjects in the Rollover Population of 270-301 receiving a more extended course of corticosteroids. Of note the Directly Enrolled subjects of 270-301 had similar total corticosteroid exposure to subjects from 270-201, while the Rollover Population (most of whom were treated after the change in approach/protocol amendment discussed above were implemented) received the longer duration and higher total dose of corticosteroids. In response to D150, the applicant clarified that the Rollover Population continued with corticosteroid as long as ALT levels had completely returned to baseline levels (different than Directly Enrolled Population).

The subjects in 270-201 received corticosteroids an average of 7.5 weeks earlier following BMN 270 infusion, while the subjects in the Rollover Population of 270-301 received more than double the total dose of corticosteroids and received corticosteroids for an average of 80 days longer than subjects in 270-201 or subjects in the Directly Enrolled Population of 270-301.

A corticosteroid decrease protocol according to the evolution of ALT was defined in clinical trials protocol.

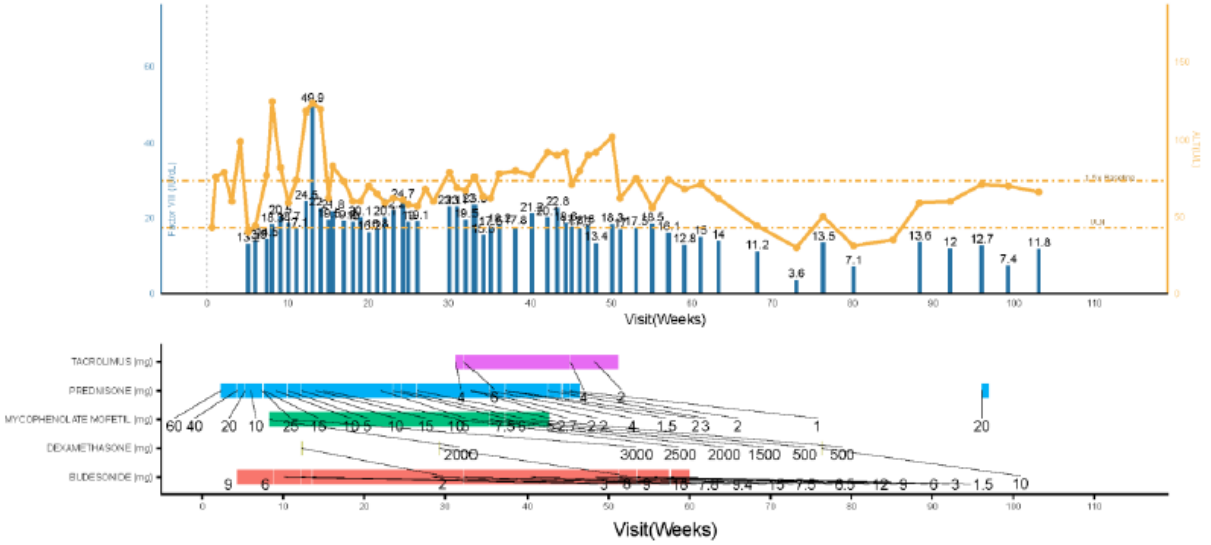
The disproportionate and limited numbers of patients who received early versus therapeutic corticosteroids make it difficult to definitively determine the best corticosteroid regimen to achieve optimal long-term FVIII expression with the least AE. No correlation of duration and total dose of corticosteroid use, ALT elevations, FVIII activity and related adverse events observed in patients who received prophylactic vs therapeutic treatment could be demonstrated by the applicant in response to D120 LoQ and D150.

Corticosteroid-related adverse events following BMN 270 administration were generally Grade 1 or 2 in intensity, manageable clinically and resolved after corticosteroid discontinuation without any long-term clinical sequelae.

Given extending the course of corticosteroids in subjects in the Rollover Population of 270-301 did not significantly impact FVIII outcomes but may have contributed to the increase in AEs/SAEs, a limited corticosteroid regimen being proposed by the Sponsor is likely to mitigate for the corticosteroid-related side effects. However, it should be taken into account the lack of long term data, the long duration of treatment needed with corticosteroids. The corticosteroid regimen, which could be proposed in the SmPC, should aim to attenuate the early inflammatory response to BMN 270 and facilitate transduction, while minimizing corticosteroid-related side effects with targeted, limited use. Given the more aggressive CS regimen in the Rollover population did not change the FVIII outcomes but contributed to corticosteroid adverse events, the applicant has proposed a shorter and optimised corticosteroid regimen in the SmPC. It should be noted that due to changes in proposed strategies, the optimal CS regimen remains to be determined. To this purpose it is noteworthy that the applicant is still willing to test a prophylactic approach with the new study 270-303 and the ongoing studies 270-203 and 270-205. Results of these studies will need to be put into perspective with the data provided via the registry, where patients will receive an optimised reactive CS regimen and with the data provided from the pivotal study.

It is noted that the majority of patients need corticosteroids for notably ALT elevations/to manage hepatic reactions.

In response to D150 LoQ, the applicant provided the whole narratives for 270-301 subjects categorised by maximum daily dose of corticosteroids and duration of use and made corresponding figures to show evolution of FVIII, transaminases and corticosteroids use (the way the presentation was to be made was determined during a dedicated clarification meeting as regards the importance of the issue). An illustration is provided as follows:



In response to D150, in the state of available data and taking into account the different modifications performed in the corticosteroids regimen in the study protocol, no trend of intrinsic or extrinsic factors of prolonged corticosteroid exposure can be observed in many approaches carried out by the applicant.

Forty (29.9%) subjects in 270-301 received immunosuppressants (IS) other than oral prednisone or prednisolone, including alternate immunosuppression agents (AIS). Approximately 40% of patients initiated IS for ALT > ULN and almost 60% started AIS to reduce ALT to baseline. In addition, most patients received AIS and corticosteroids concomitantly. These agents included tacrolimus (24 subjects, 17.9%), mycophenolate (13 subjects, 9.7%), methylprednisolone (7 subjects, 5.2%), and budesonide (6 subjects, 4.5%) (some subjects may have received more than one type of AIS). There were no significant adverse event due to AIS, except one SAE of CMV pneumonia reported as related to use of tacrolimus that resolved with many treatments without clinical sequelae.

In view of these safety data observed and noting that aggressive immunosuppression does not seem to modify efficacy of BMN 270 or recurrence of ALT elevations, the number of patients to receive AIS should be limited only to patients presenting tolerance issues related to corticosteroids or ineffectiveness of oral corticosteroids.

No subjects in 270-201, 270-302 and 270-203 have initiated corticosteroids since the MAA data cutoff, and no new corticosteroid-related AEs have been reported.

Infusion-associated events, including infusion-related reactions, hypersensitivity, anaphylactic, or anaphylactoid reactions

o Infusion-associated events, defined as TEAEs occurring during BMN 270 infusion or within 48 hours post-infusion

o Infusion-related reactions, defined as TEAEs occurring during BMN 270 infusion or within 6 hours post infusion

o Systemic hypersensitivity (Hypersensitivity [SMQ] – narrow scope) during BMN 270 infusion or within 48 hours post-infusion

o Anaphylactic or anaphylactoid reactions (Anaphylactic reaction [SMQ] – algorithmic) during BMN 270 infusion or within 48 hours post-infusion

Less than 40% of patients (59/151 in the All-Treated Population, i.e. 39.1% and 53/141 in the Proposed Label Population, i.e.37.6%) experienced at least 1 potential infusion-associated event. The most commonly reported infusion-associated events included nausea (20 patients (13.2%) in the All Treated Population / 19 patients (13.5%) in the Proposed Label Population), fatigue (11 patients (7.3%) in the All Treated Population / 10 patients (7.1%) in the Proposed Label Population), headache (8 patients in the All Treated Population and in the Proposed Label Population), diarrhoea (4 patients in the All Treated Population and in the Proposed Label Population), dizziness, pruritus and vomiting (in 3 patients in the All Treated Population and in the Proposed Label Population). These events appeared in the first 48 hours following BMN 270 administration.

Of the 91 infusion-associated events reported in the Proposed Label Population, 88 (96.7%) were Grade 1 or Grade 2, and 3 (3.3%) Grade 3 (hypersensitivity, hypertension, and anaphylactic reaction). 55 (60.4%) were assessed as related to treatment with BMN 270.

All 16 infusion-related reactions reported in 11 patients, during or within 6 hours after the end of the BMN 270 infusion, including 4 serious events in 3 patients (2 Grade 2 in one patient (maculopapular rash and presyncope) and 2 Grade 3 (hypersensitivity and anaphylactic reaction)), are resolved without sequelea. These serious events, which led to the temporary interruption of the BMN 270, later restarted and completed. In the All-Treated Population, all subjects successfully completed their prescribed BMN 270 infusion, and none of the infusion-associated events led to permanent discontinuation or early termination of the infusion.

Following these serious events observed in clinical trial 270-301, the protocol was amended to change the starting infusion rate to 1 mL/min instead of 4 mL/min. In the Proposed Label Population, 16 patients (11.3%) were dosed with the original starting infusion rate of 4 mL/min, and 125 patients (88.7%) have been dosed with the amended 1 mL/min starting infusion rate. 36% patients (45/125) in the 1 mL/min population experienced at least one infusion related reaction, which is lower than 50% patients (8/16) in the 4 mL/min population. A lower incidence of serious infusion reactions in the 1 mL/min population (1/125 patients [0.8%]) was also seen than in the 4 mL/min population (1/16 patients [6.3%]).

Thromboembolic events

o Embolic and thrombotic events (SMQ) for entire study period

o TEAEs suggestive of symptoms associated with thromboembolic events occurring during periods where a subject's FVIII activity is > 150 IU/dL.

No events or symptoms suggestive of thromboembolism were observed. However, a potential increased risk of thrombosis associated with increased levels of FVIII due to overexpression of the transgene resulting in supra-physiological FVIII activity levels (> 150%) could not be excluded. The risk of thromboembolism is at this purpose considered as an important potential risk.

It is noted that transient FVIII activity levels above the ULN was observed in 57% (4/7) patients in the 6E13 vg/kg cohort in clinical trial 270-201 and 28% (38/134) patients in clinical trial 270-301. Some remained above the ULN at the resubmission data cut-off. However, no thromboembolic events have arisen in the small number of patients who had supraphysiologic levels of FVIII for an extended period of time. While no thromboembolic events have been reported following BMN 270 infusion, overexpression of the transgene resulting in sustained supra-physiological FVIII activity levels (> 150%) is a theoretical safety concern due to possible increased risk of thrombosis associated with increased levels of FVIII.

Only one patient treated in 270-301 study experienced cardiologist evaluated non-cardiac chest pain (resolved to date) concurrently with an FVIII activity level >300 IU/dL. Anticoagulants were then initiated on the safe side. As of Week 104, this patient is still on anticoagulants, as a precaution. At the data cut-off date of 15 November 2021, no other subjects received antiplatelet or anticoagulation therapy prompted by an observed elevation in FVIII activity.

Although history of venous or arterial thrombotic/thromboembolic events or significant thrombophilia was an exclusion criterion in clinical trials, no explicit exclusion criteria related to clinical risk factors for thromboembolism was mentioned in BMN 270 clinical trials. The applicant clarified that some patients who could be considered presenting risk factors for thromboembolism (hypertension, hypercholesterolemia, hyperlipidemia, diabetes, obesity and coronary artery disease) were enrolled across clinical trials 270-201 and 270-301. Smoking history was neither part of the exclusion criteria or specifically collected in the studies. None of these subjects experienced a thromboembolic event or symptoms suggestive of thromboembolism. At the resubmission date cut-off, in 270-301, 3 subjects (2.2%) were identified as having AEs that met the search strategy for symptoms potentially suggestive of thromboembolic TEAEs occurring during a period of elevated FVIII activity levels. The four events were all Grade 1 (3 events) or Grade 2 (1 event) headache (including Grade 1 and Grade 2 events in 1 subject) and were not considered related to underlying thrombosis. The events had a duration of 1-40 days, and 3 of the 4 were treated with routine pain medication. None of the subjects have developed other symptoms or complications related to thrombosis.

Development of anti-FVIII inhibitors (neutralizing antibodies) were assessed by reports using the MedDRA PT "Anti factor VIII antibody positive"

No patient had developed neutralizing antibody to FVIII.

2.6.8.3. Serious adverse event/deaths/other significant events

Fifty-nine SAEs (in 32 subjects) have been reported across all BMN 270 clinical studies including 4 new SAEs (in 4 subjects) in 270-301 and two new SAEs in 270-201 detailed in response to D120 LoQ. All 7 SAEs were assessed as unrelated to BMN 270 and immunosuppressant use.

A late-breaking event of acinic cell carcinoma of the parotid gland was reported in a male patient at day 2050 (approx. 5 years) after having been dosed at 6E13 vg/kg in study 270-201 after the data cut-off for the Year 5 270-201 analysis. This patient underwent right parotidectomy with lymph node dissection in December 2021. Since then, the patient is clinically well. In response to the CAT List of Questions, the applicant presented the results of different genomic analyses conducted on DNA extracted from excised parotid gland tissue (tumour tissue and adjacent healthy tissue). The performed dedicated investigations of resected parotid tissue did not establish any association of BMN 270 integrations, nor any other genomic lesions, with this case of acinic cell carcinoma of the parotid gland. Based on these analyses, this event of acinic cell carcinoma is considered likely unrelated to BMN 270, which is consistent with previous assessments by the Principal Investigator and the DMC. Nevertheless, a small number of BMN 270 integrations were identified in each of the four parotid gland DNA samples: 26 and 33 identified BMN 270 IS in the two tumour tissue DNA samples and 54 and 37 identified BMN 270 IS in the two healthy tissue DNA samples. It is a lower BMN 270 vector quantities in the parotid gland compared to liver, as expected.

At the time of the data cut-off, forty-six of the 53 SAEs occurred in subjects in the Proposed Label Population. No SAE was reported more than twice; SAEs reported in 2 subjects included ALT increased, arthropathy, diarrhoea, gastroenteritis, haemophilic arthropathy, hypersensitivity, and rectal haemorrhage. The MedDRA SOCs with the most reported SAEs included Injury, Poisoning and Procedural Complications (9 events); Infections and Infestations (7 events); and Musculoskeletal and

Connective Tissue Disorders (6 events). Most SAEs (32/53, 60.4%) were Grade 3 in severity; 14 events (26.4%) were Grade 2, 5 events (9.4%) were Grade 4, and 2 events (3.8%) were Grade 1. The most commonly reported SAEs with a severity of Grade 3 or higher were depression/major depression (3 SAEs in 1 subject), ALT increased (2 SAEs in 2 subjects), diarrhoea (2 SAEs in 2 subjects), gastroenteritis (2 SAEs in 2 subjects), cataract (2 SAEs in 1 subject), and skin laceration (2 SAEs in 1 subject).

Six SAEs (in 3 subjects) were assessed by Investigators as possibly related to use of corticosteroids (single events of rectal haemorrhage, pneumonia, influenza A virus test positive, hypertension, steroid diabetes, and diabetes mellitus).

Only 1 SAE was reported after Week 1 but before Week 15.

The reported SAEs include 8 events assessed as related to treatment with BMN 270 by the investigators, with 2 new serious Grade 3 ALT increased:

• 270-201 (4E13 vg/kg) – Grade 2 pyrexia

• 270-203 (6E13 vg/kg, AAV5 TAb+) – Grade 2 hypersensitivity

• 270-301 (6E13 vg/kg) – Grade 2 maculo-papular rash and Grade 2 presyncope

• 270-301 (6E13 vg/kg) – Grade 3 hypersensitivity

• 270-301 (6E13 vg/kg) – Grade 3 ALT increased

• 270-301 (6E13 vg/kg) – Grade 3 anaphylactic reaction

• 270-301 (6E13 vg/kg) – Grade 3 ALT increased

All treatment-related SAEs had resolved as of the data cut. No subject withdrew from the study or was unable to complete his BMN 270 infusion as a result of an SAE.

2.6.8.4. Laboratory findings

In addition to ALT elevations discussed above, at the time of the resubmission data cut-off, 122 of 151 patients (80.8%) in the All-Treated Population and 113 of 141 patients (80.1%) in the Proposed Label Population had at least one ALT elevation above ULN, after BMN 270 administration. Patients enrolled in 6E13 vg/kg cohort of 270-201 developed ALT elevation about 3 weeks later than patients in 270-301, generally once the first course of corticosteroids was being tapered, and experienced lower peak elevations in ALT (75.7 U/L) than subjects in 270-301 (112.5 U/L). The difference in the ALT profile could be attributed to the difference in the protocol-specified corticosteroid regimens in place in those clinical trials, including the use of prophylactic corticosteroids in 270-201.

One hundred and fourteen patients in the All-Treated Population (including 99 patients treated in 270-301) had ALT elevations that were either ≥ 1.5x ULN or (> ULN and > 2x baseline) reported as an EOSI. One hundred and twenty-six patients (83.4%) in the All-Treated Population (including 124 patients treated in 270-301) had ALT elevations that were ≥ 1.5x Baseline or > ULN. 13 patients (8.6%) in the All-Treated Population (12 patients in 270-301 and 1 in 270-302) experienced ALT > 5x ULN. All were resolved.

In response to D120 LoQ, one new Grade 1 ALT elevation was reported in 270-201 and 67 new AEs of ALT elevation in 270-301 (61 Grade 1, 5 Grade 2, and 1 Grade 3). 34 (50.7%) assessed by the Investigators as unrelated to BMN 270 treatment and related to life events such as strenuous exercise or alcohol consumption.

Among other liver function tests, at the time of the resubmission data cut-off, 103 of the 151 (68.2%) of the subjects in the *All-Treated Population* had AST elevations during the course of the study. In 56 subjects the AST elevation was reported as an AE. Ten subjects (6.6%) had Grade 3 AST elevations (CTCAE definition > 5x to < 20x ULN). No subject had more than 1 episode of Grade 3 AST elevation. In most cases, the AST elevation accompanied elevations in other blood markers (such as ALT or CPK); in one instance, a subject had an isolated ALT elevation a day after taking acetaminophen. It is noted that transient increases in aminotransferases had no major impacts upon long-term liver function, which remained stable over time in all subjects.

Shifts from normal at baseline to abnormal during the study were limited and transient for other liver tests (total bilirubin, elevation of alkaline phosphatase and GGT elevation). All the liver functions test changes events were resolved with no long-term sequelae.

No consistent or clinically meaningful changes from Baseline in hematology results were evident with no significant changes in the mean or median changes. Few patients presented abnormalities in the results in hematology: anaemia, increase in haemoglobin, decrease / increase in the number of lymphocytes, decrease in the number of neutrophils, decrease in the number of platelets, decrease in the number of white blood cells. These events were Grade 1 or Grade 2 or Grade 3, and were resolved without clinical sequelae.

The chemistry test results that most frequently shifted from normal at Baseline to out of range values at any time post-Baseline were ALT, AST, CPK, LDH, GGT, cholesterol, phosphate, and glucose. Increase in cholesterol and decline in phosphate observed in less than 30% of all patients treated with BMN 270 were considered clinically insignificant. AEs were observed in 12/67 patients (16 increase in LDH), in 19/45 patients (36 increase in CPK) and in 4 patients (7 hyperglycemia events). All events were resolved. Several clinical chemistry events occurred concurrently with ALT elevations or with exercise or associated with concomitant use of corticosteroids. Most were limited and resolved without treatment. No patient was withdrawn from a BMN 270 clinical trial as a result of a clinically significant laboratory abnormality.

No consistent or clinically meaningful changes from Baseline in urinalysis results was evident.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable

2.6.8.6. Safety in special populations

No major difference could be noted in the All-Treated Population comparing White subpopulation (110 patients [72.8%]) and non-White subpopulations (41 patients [27.2%] including Black, Asian, and Pacific Islander). The exposure-adjusted event rate in the White patients in the All-Treated Population was higher (10.93 TEAEs/patient-year, compared with 8.42 TEAEs/patient-year in other patients), though non-White patients had an SAE rate that was twice than of the White patients (0.36 SAEs/year for non-White patients vs. 0.17 for White patients), which can be attributed to a higher dose of corticosteroids. 10 non-White patients (5 Black or African-American, 4 Asian, and 1 race not provided due to privacy reasons) experienced 22 total SAEs. All but 2 SAEs (hypersensitivity in the patient with no reported race, and anaphylactic reaction in an Asian patient) were assessed as not related to treatment with BMN 270. No difference was noted in the event rates of treatment-related TEAEs or EOSI.

It is noted that 13/151 (8.6%) patients who received 6E13 vg/kg BMN 270 were over 50 years of age at the time of administration, including only one patient who did not have a serious Grade 3 or higher AEs. Apart from this patient, none of the 12/13 patients in the ≥ 50 years age group were older than

58 years of age. The event rate of TEAES is higher for the age 50+ patients (15.58 AEs/year) than the two other largest groups (9.54 AEs/year for the age 18-30 patients and 10.33 AEs/year for the age 30-50 patients). The SAE rate was also highest in the age 50+ patients, as was the rate of Grade 3 AEs compared with rates observed in the other groups. SAE observed in the age 50+ population not related to BMN 270, were largely age-related issues or related to other concomitant medications. No difference was noted in the event rates of treatment-related AEs and of EOSI.

As of the recent data cut-off, 7 partner pregnancies had been reported (4 in 270-301, 3 in 270-201), 6 after BMN 270 infusion (40 months after BMN 270 administration, almost 1 years after BMN 270 administration, more than 4 years after the subject cleared DNA in semen, 1 year after the subject cleared vector DNA from semen, 12 months after the 3rd clear semen sample, between the first and the second negative semen samples, ie 38 weeks after infusion) and one prior to BMN 270 infusion, all of which resulted in term deliveries without clinical sequelae. The applicant specified that the female partners and newborns from patients treated in clinical trials with BMN 270 were not investigated for vector transmission. The conclusion that there is a negligible risk of horizontal and vertical transmission at time of pregnancy is endorsed considering that there is a negligible risk of horizontal and vertical transmission at time of pregnancy. Indeed, the six female partners became pregnant more than 9 months after BMN 270 administration.

MedDRA Terms	Age <65 Incidences (N=150), n(%)	Age 65-74 Incidences (N=1), n(%)	Age 75-84 number (N=0)	Age 85+ number (N=0)
Total AEs	150 (100.0)	1 (100.0)	N/A	N/A
Total SAEs	32 (21.3)	0	N/A	N/A
• Fatal	1 (0.7)	0	N/A	N/A
• Hospitalisation/prolong existing hospitalisation	32 (21.3)	0	N/A	N/A
• Life-threatening	3 (2.0)	0	N/A	N/A
• Disability/incapacity	1 (0.7)	0	N/A	N/A
• Other (medically significant)	14 (9.3)	0	N/A	N/A
AEs leading to drop-out	0	0	N/A	N/A
Psychiatric disorders	59 (39.3)	0	N/A	N/A
Nervous system disorders	82 (54.7)	0	N/A	N/A
Accidents and injuries	67 (44.7)	0	N/A	N/A
Cardiac disorders	10 (6.7)	0	N/A	N/A
Vascular disorders	20 (13.3)	0	N/A	N/A
Infections and infestations	122 (81.3)	1 (100.0)	N/A	N/A
Anticholinergic syndrome	0	0	N/A	N/A
Quality of life decreased at Week 104 from Baseline	29 (20.9)	0	N/A	N/A
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	26 (17.3)	0	N/A	N/A
AEs appearing more frequently in older patients		Abdominal pain upper, Adenoma benign, Arthralgia, Asthenia, Back pain, Conjunctivitis, Cough, Gastrointestinal dysplasia, Gastrooesophageal reflux disease, Musculoskeletal chest pain, Pain, Pruritus, Pyrexia	N/A	N/A

2.6.8.7. Immunological events

Pre-existing antibody and other inhibitors against the viral vector may interfere with receptor binding, vector uptake and lead to reduced transduction. To avoid any potential safety or efficacy-related consequences from AAV5 immunogenicity, a screening strategy for detection of pre-existing AAV5 antibody was used to select subjects for all BMN 270 clinical trials. This screening strategy evolved over time, resulting in development of the AAV5 TAb assay as a companion diagnostic in partnership with ARUP Laboratories (Salt Lake City, USA). AAV5 TAb positive subjects were excluded from all BMN

270 clinical studies, except from 270-203, which was specifically designed to assess efficacy and safety in subjects with pre-existing AAV5 TAb titers.

AAV5 TAb positive patients were excluded from all BMN 270 clinical trials (except from 270-203 with only one patient treated so far) and all treated patients developed an immune response against the AAV5 capsid. It is noted that a moderate positive correlation exists between AAV5 capsid-specific cellular immune responses and (elevated) ALT levels, sometimes reported as AEs. These results suggest that AAV5-specific cellular immune responses may be one factor contributing to transient ALT elevations in some subjects, although causality cannot be concluded. The early occurrence of these responses suggests that potentially pro-inflammatory events may be occurring prior to ALT elevations, of relevance when considering the proposed timing of early corticosteroid initiation. These observations should be verified in a larger number of patients treated with BMN 270. Clinical relevance is unknown and remains to be substantiated. A dedicated study is ongoing in patients with pre-existing AAV5 immunity.

Concerning the potential immunogenicity with development of antibodies to transgene SQ FVIII protein that the body synthesizes after treatment with BMN 270, no patient dosed with BMN 270 developed FVIII inhibitors so far (neutralizing antibody to FVIII). This concern is considered as an important potential risk especially in patients with less than 150 exposure days to FVIII concentrates or cryoprecipitate given the limited clinical experience. Moreover, sporadic positive FVIII TAb results and FVIII cellular immune responses were detected in a limited number of patients, without any corresponding impact to ALT levels, FVIII activity, or inhibitor positively. A dedicated study has recently been initiated in patients with a history of FVIII inhibitors. The therapeutic indication proposed in the SmPC has been adequately revised to exclude categorically patients with history of FVIII inhibitors in reflect of the population studied in the clinical trials. Section 4.4 of the SmPC informs that "*Patients who have or had inhibitors (neutralising antibodies) to factor VIII were excluded from participation in the clinical studies. It is not known whether or to what extent such inhibitors affect the safety or effectiveness of ROCTAVIAN*" and a monitoring of patients for the development of factor VIII inhibitors by appropriate clinical observations and laboratory tests will be performed after administration of ROCATVIAN.

2.6.8.8. Safety related to drug-drug interactions and other interactions

No formal studies of drug-drug or drug-disease interactions were performed.

The SmPC warns healthcare professionals against co-administration with the potentially hepatotoxic medications/agents, that they may reduce the effectiveness of Roctavian and increase the risk for more serious hepatic reactions, particularly during the first year following Roctavian administration. The applicant proposed to include statements in sections 4.5 and 4.4 of the SmPC to inform healthcare professionals about the risk of the concomitant use of BMN 270 with potentially hepatotoxic medications and to advise healthcare professionals to evaluate the patient´s concomitant use of medications as well as hepatic status prior administration of BMN 270, to evaluate the concomitant use of medications after administration of BMN 270 (in particular during the first year) and if newly drugs are administered to closely monitor the patient´s ALT levels and FVIII activity. The proposed SmPC statements are considered acceptable to inform and advise healthcare professionals about these risks of concomitant use of BMN 270 with potentially hepatotoxic drugs.

Regarding the observation of two cases of reduced FVIII activity following introduction of isotretinoin initiated at W60 and extroamphetamine/amphetamine initiated at W42, the SmPC has been changed to monitor patients in case of any newly introduced medications. Conservatively, the SmPC now recommends to close monitor patients (FVIII activity and ALT levels) when a new concomitant medication is started, after BMN 270 administration and to evaluate the need for adjustments.

Finally, the SmPC provides guidance on monitoring medications and limiting use of potential hepatotoxic medications, or other hepatotoxic agents (including alcohol, potentially hepatotoxic herbal products and nutritional supplements) or medications which may interfere with metabolism of corticosteroids (eg, agents that induce or inhibit cytochrome P450 3A4) and may decrease the effectiveness of the corticosteroid regimen and subsequently may lead to adverse ALT outcomes. It was the case for one patient treated in clinical trial 270-301 on concurrent carbamazepine for seizure disorder who experienced a Grade 3 ALT elevation after oral corticosteroids, which responded to IV methylprednisolone).

It is noted that an HIV-positive patient experienced a possible hepatotoxicity interaction between BMN 270 and a concomitantly administered hepatotoxic drug (Efavirenz) in clinical trial 270-302. This subject on a long-term, highly active anti-retroviral treatment (HAART) regimen to control HIV infection experienced asymptomatic Grade 3 elevations of ALT, AST and GGT and a Grade 1 elevation of serum bilirubin. The reaction did not respond to corticosteroid treatment but responded to withdrawal of efavirenz, and resolved after his antiretroviral therapy regimen was changed to a regimen without efavirenz. The patient later reverted to prophylactic use of FVIII concentrates/hemostatic agents. No hepatotoxicity has been observed in subjects who received lamivudine (which was later removed from the list of prohibited medications in BMN 270 clinical study protocols), or in HIV-positive subjects receiving other HAART medications (such as emtricitabine, rilpivirine, tenofovir, darunavir, dolutegravir, and ritonavir). These results suggest that HIV infection, in and of itself, does not confer an increased risk of hepatotoxicity following BMN 270 infusion. The applicant stated that underlying risk of potential interactions with hepatotoxic medications is covered under identified risk of hepatotoxicity by avoidance of concomitant medications that are potentially hepatotoxic, due to potential reduction of effectiveness of treatment and increase the risk for more serious hepatic reactions, particularly during the first year after Roctavian administration (sections 4.4, 4.5, 4.8 of the SmPC). The applicant considers that HIV-infected patients could be treated with Roctavian provided they are well controlled for their HIV infection and are on non hepatotoxic anti-retroviral regimen. A specific warning is provided in the SmPC. The management of hepatotoxicity in HIV-positive patients has been better described in the SmPC. Regarding the enrolment of HIV-infected patients in ongoing studies, the applicant replied that he was working to amend protocols for clinical trials with ongoing enrollment (270-203, 270-205 and 270-303) in order to allow the inclusion of HIV-infected patients by July 2021. The applicant should pay particular attention and review of HIV-infected patients who will enroll in the post-marketing observational study.

2.6.8.9. Discontinuation due to adverse events

As of the data cut-off, no patients have withdrawn from any BMN 270 study. All subjects completed the BMN 270 infusion and remained in the study except one patient in 270-301 lost to follow-up at Week 66 post-infusion.

No patients have required permanent termination of an infusion prior to completion due to AEs. Three infusion-related reactions (hypersensitivity, maculo-papular rash and anaphylactic reaction) have been reported in study 270-301 that led to temporary interruption of the BMN 270 infusion. Both events occurred within 1 hour of initiating the BMN 270 infusion. Please refer to the section Adverse events.

2.6.8.10. Post marketing experience

Not applicable as BMN 270 is currently not marketed in any country.

2.6.9. Discussion on clinical safety

Safety database

The safety data comes from 4 clinical trials who patients received any dose of BMN 270, similarly to the withdrawn MAA. In the current application, safety data are based on 151 patients (All Treated Population), including 141 patients (Proposed Label Population) as intended population (AAV5 Tab-negative) who received BMN 270 at the therapeutic dose (6E13 vg/kg), while data on only 49 patients were presented in the withdrawn application, i.e. more than 3.5 times more exposed patients in this current application.

In general, the safety profile of BMN 270 could be considered as acceptable with some uncertainties not yet elucidated. It is difficult to conclude because of multiple factors: lack of a control arm, limited data on long term follow-up and impact of additional different corticosteroid treatment. Additional uncertainly arises from the fact that patients were treated with different batches manufacturing by different processes with different transgene activity. A wide range of clinical response with a high variability of FVIII activity levels achieved can be observed (even in patients dosed with the same drug product lot). In response to D120 LoQ, the applicant demonstrated that there is no clear association between the manufacturing process of BMN 270 or the clinical batch used and the nature/profile of AEs reported. No trends were observed. The safety profile of patients treated at the therapeutic dose is generally comparable regardless of the clinical batch/manufacturing process used.

The number of patients treated at the therapeutic dose with a relevant duration of long-term data may be questionable. One subject in 270-301 withdrew at Week 66, and one subject died at Week 95; all other subjects (149/151, 98.7%) have completed the Week 104 post-infusion follow-up, while 33/151 (21.9%) had follow-up for more than 3 years, 15/151 (10.0%) for more than 4 years, and 8/151 (5.3%) for more than 5 years as of the individual study data cutoff dates. The total exposure as of the data cutoffs for all studies was 391.8 patient-years, including 352.8 patient-years at the 6E13 vg/kg proposed label dose.

Safety profile

All patients treated with BMN 270 experienced at least one TEAE, irrespective of causal relationship to BMN 270 infusion. At the time of resubmission, a majority of patients experienced only Grade 1 (mild) or Grade 2 (moderate) TEAEs. Grade 3 events occurred in less than 30% of patients. 2 patients reported 5 Grade 4 events and 1 Grade 5 event, all assessed as unrelated to BMN 270. Some events were assessed as related to BMN 270 or immunosuppressant therapy (including corticosteroid treatment).

More than 20% of patients treated with BMN 270 experienced at least 1 SAE.

The incidence of AE was higher during the first 26 weeks after the infusion (87.9%) and dropped considerably after week 52 (7-12%). Infusion-associated events occurred within the first 4 weeks after infusion. Hepatic reactions occurred most commonly between Weeks 4-26 and declined steadily after. Less than 40% of patients exposed to BMN 270 experienced infusion-associated events and more than 85% of patients exposed to BMN 270 experienced hepatic reactions.

All patients successfully completed their full-dose infusion of BMN 270; no infusions required permanent termination prior to completion due to AEs. No patient discontinued study treatment during BMN 270 infusion or was withdrawn from a study as a result of an AE.

Hepatotoxicity

In the resubmission data cut-off, in the Proposed Label Population, elevations in ALT meeting the protocol-defined EOSI threshold were reported in 122 patients (86.5%) (115 patients from 270-301) following BMN 270 administration. The critical aspect regarding this AE is the need to a corticosteroid treatment.

Regarding the duration of events in the Proposed Label Population, the mean (SD) event duration was 48.9 (79.5) days (median 16 days; range 1-488 days). The applicant stated that ALT elevations responded rapidly to corticosteroid initiation. However it could be noted that the event was considered resolved more than 30 days after the AE onset in many patients and up to 177 days (patient in 270-201) or 488 days (patient in 270-301) despite the use of corticosteroids. It is also noted that some subjects had another events despite the ongoing corticosteroid treatment and some need addition of others IS. In view of safety clinical data, it is difficult to evaluate the hepatoxicity events and the consequence of corticosteroid use.

One hundred and thirteen (92.6%) patients (106 from 270-301) received oral corticosteroids for ALT elevations. Some patients did not receive corticosteroids despite reported events of ALT elevations. The management of treatment evolved in clinical trials and different approaches to corticosteroid administration were employed in BMN 270 clinical trials: corticosteroid if the LFTs were abnormal, to a prophylactic corticosteroid treatment (starting at week 3) before to remove the requirement for prophylactic oral corticosteroids due to AE observed. The median duration of corticosteroid for ALT elevations in the Proposed Label Population is 32.4 weeks (min-max: 3-79 weeks). However, it is noted that some patients treated with 6E13 vg/mL BMN 270 had been treated for at least 6 months with corticosteroids. Dependence of corticosteroids could be disputable for these patients.

Due to limited numbers of patients who received early/prophylactic versus therapeutic corticosteroids, it is difficult to determine with certainty the best corticosteroid regimen to facilitate transduction, achieve optimal long-term FVIII expression and reduce the early inflammatory response to BMN 270 with the least AE/SAE. Given the high dose and prolonged treatment of corticosteroids observed in the Rollover Population of 270-301, which may have contributed to increase the AEs/SAEs, but did not change the FVIII outcomes, the applicant proposes in response to D120 LoQ and completes in response to D150 a shorter and optimised corticosteroid regimen in the SmPC. However, it should be noted that due to changes in proposed strategies, the optimal regimen of CS cannot be determined. It is noteworthy that the applicant is still willing to substantiate a prophylactic approach through a new study 270-303 and the ongoing studies 270-203 and 270-205. Results of these studies will need to be put into perspective with the data provided via the registry, where patients will receive an optimised reactive CS regimen and with the data provided from the pivotal study

Finally, hepatotoxicity is considered as an important identified risks with some requirements concerning this risk including in the SmPC or in additional mitigation measures.

Infusion reactions

Less than 40% of patients in the All-Treated Population and 37.6% in the Proposed Label Population) experienced at least 1 potential infusion-associated event. These events appeared in the first 48 hours following BMN 270 administration. Of the 91 infusion-associated events reported in the Proposed Label Population, 88 (96.7%) were Grade 1 or Grade 2.

All 16 infusion-related reactions reported in 11 patients, during or within 6 hours after the end of the BMN 270 infusion, including 4 serious events in 3 patients and 2 Grade 3 were resolved without sequelae. These serious events, which led to the temporary interruption of the BMN 270, later restarted and completed.

Following these serious events observed in clinical trial 270-301, the protocol was amended to change the starting infusion rate to 1 mL/min instead of 4 mL/min. A lower incidence of serious infusion reactions in the 1 mL/min population (1/125 patients [0.8%]) than in the 4 mL/min population (1/16 patients [6.3%]) could be observed.

Finally, infusion reactions are considered as an important identified risk. In addition to recommendations on the infusion rate of BMN 270 (1 mL/min) and management in case for an

infusion-related reaction (decrease the rate or stop the infusion and appropriate treatment), mitigation measures are included in the SmPC.

Thromboembolic events

No event or symptom suggestive of thromboembolism was reported. However, a potential increased risk of thrombosis associated with increased levels of FVIII due to overexpression of the transgene resulting in supra-physiological FVIII activity levels (> 150%) could not be excluded. The risk of thromboembolism is therefore considered to be an important potential risk.

Section 4.4 of the SmPC highlights the lack of experience in patients with a relevant history of venous or arterial thrombotic/thromboembolic events or know history of thrombophilia. The Patient Card and Healthcare Professional Guide were amended satisfactorily in accordance to the SmPC concerning the risk of thromboembolism.

Development of anti-FVIII inhibitors (neutralizing antibodies)

No patient in any BMN 270 study developed neutralizing antibody to FVIII.

This immune response to FVIII with development of antibodies to transgene SQ FVIII protein is considered as an important potential risk.

As proposed in the SmPC section 4.4, healthcare professionals will be informed that "Patients who have or had inhibitors (neutralising antibodies) to factor VIII were excluded from participation in the clinical studies. It is not known whether or to what extent such inhibitors affect the safety or effectiveness of ROCTAVIAN" and a monitoring of patients for the development of factor VIII inhibitors by appropriate clinical observations and laboratory tests will be performed after administration of ROCATVIAN.

It could be noted that patients with pre-existing FVIII inhibitors will be clearly excluded from treatment with Roctavian in line with what has been done in clinical trials 270-201 and 270-301.

Serious adverse events and deaths

Fifty nine SAEs were observed in 32 patients in the All Treated Population, and less than 20% of the patients treated at the therapeutic dose experienced at least one SAE.

Only 5 Grade 4 SAEs in 2 patients and 1 Grade 5 SAE were reported, all assessed as unrelated to BMN 270.

Only 8 SAEs in 7 patients were assessed as related to treatment with BMN 270 with 2 new serious Grade 3 ALT increased. 6 serious infusion-associated events (Grade 2 hypersensitivity, Grade 2 maculopapular rash and presyncope, Grade 3 hypersensitivity) in 5 patients occurred on the day of the BMN 270 administration (but Grade 2 pyrexia that appeared at D2), with an initial infusion of 4 mL/min except Grade 3 anaphylactic reaction. All were resolved as of data cut-off.

One subject receiving 6E13 vg/kg BMN 270 died of suicide after the data cutoff for this MA (day 669 post-infusion). The subject had a long history of severe depression and his death was regarded as unrelated to BMN 270.

Laboratory findings

After BMN 270 administration, 122 of 151 patients (80.8%) in the All-Treated Population and 113 of 141 patients (80.1%) in the Proposed Label Population had at least one ALT elevation above ULN at the resubmission data cut-off. It could be noted that patients enrolled in 6E13 vg/kg cohort of 270-201 developed ALT elevation about 3 weeks later than patients in 270-301, generally once the first course of corticosteroids was being tapered, and experienced lower peak elevations in ALT (75.7 U/L) than subjects in 270-301 (112.5 U/L). The difference in the ALT profile could be attributed to the difference

in the protocol-specified corticosteroid regimens in place in those clinical trials, including the use of prophylactic corticosteroids in 270-201.

Among other liver function tests, at the resubmission data cut-off, 103 of 151 (68.2%) patients in the All-Treated Population had AST elevations and generally occurred concurrent with ALT elevations. In more than 50% of patients who experienced AST elevations, these events were reported as AEs. It could be noted that transient increases in aminotransferases had no impacts upon long-term liver function, which should be confirmed by updated safety data from longer-term patient follow-up.

Few patients presented abnormalities in the results in hematology: anaemia, increase in haemoglobin, decrease / increase in the number of lymphocytes, decrease in the number of neutrophils, decrease in the number of platelets, decrease in the number of white blood cells. These events were Grade 1 or Grade 2 or Grade 3, and were resolved without clinical sequelae.

Increase in cholesterol and decline in phosphate observed in less than 30% of all patients treated with BMN 270 were considered clinically insignificant. At the resubmission data cut-off, AEs were observed in 12/67 patients (16 increase in LDH), in 19/45 patients (36 increase in CPK) and in 4 patients (7 hyperglycemia events). All events were resolved, except 3 increases in CPK and 2 hyperglycemia events, which were ongoing at the resubmission data cut-off. Several clinical chemistry events occurred concurrently with ALT elevations or with exercise or associated with concomitant use of corticosteroids. Most were limited and resolved without treatment. No patient withdrew from a BMN 270 clinical trial as a result of a clinically significant laboratory abnormality.

No consistent or clinically meaningful change from Baseline in urinalysis results was evident.

Risk of Insertional mutagenesis

Vector integration at low level was observed in the integration site analysis study performed on liver biopsies of cynomolgous monkeys showing BMN 270 integration into hepatocytes genome. While AAV vectors are not expected to integrate their genome in host cells at high frequency, all integration events could contribute to tumoral transformation. Insertional mutagenesis by AAV vectors has been identified as an important potential risk.

In response to D150, the applicant provided the requested integration site analysis showing that vector integration into human liver was clearly detected in all five liver biopsies available from 5 different subjects. The number of integration sites ranged from 182 to 778 per sample, which is comparable to what has been observed in the NHP study. To better understand notably the pattern of the integration site, the results of the new liver biopsy sub-studies should be submitted once available as post-authorisation measure.

It is noteworthy that a case of acinic cell parotid carcinoma has occurred in male patient at day 2050 (approx. 5 years) after having been dosed at 6E13 vg/kg in study 270-201. It has been noted that the conclusion of the applicant supported by a DMC consulted is that the risk of vector elements contributing to tumorigenesis is low. The applicant has initiated dedicated investigations to further assess the potential causality of BMN 270. Results of genomic analyses to investigate potential genetic drivers of the malignancy were provided, in response to D150 and did not establish any association of BMN 270 integrations, nor any other genomic lesions, with this case of acinic cell carcinoma of the parotid gland. Based on these analyses, this event of acinic cell carcinoma is likely unrelated to BMN 270, which is consistent with previous assessments by the Principal Investigator and the independent DMC consulted. The risk of rAAV integration contributing to tumorigenesis for this case therefore seems low with BMN 270, which is rather reassuring. Nevertheless, a small number of BMN 270 integrations were identified in each of the four parotid gland DNA samples. It is a lower BMN 270 vector quantities in the parotid gland compared to liver, as expected. Data are mentioned in section 4.4 of the SmPC.

Combined, these findings show that Roctavian integrates into human DNA in different cells of the body Based on currently available data, no cases of malignancies associated with Roctavian treatment have been reported. Notably, however, the clinical relevance of these findings is unknown, as potential malignancies that might arise due to vector integration may only develop over time.

Based on the available data, the CAT is of the opinion that the risk of malignancy in relation to the identified DNA vector integration cannot be currently substantiated in human patients treated with rAAV vectors (since requiring long term data), but must be carefully considered. As the risk of malignancy associated with identified DNA integration Roctavian is not established, albeit potential, it hence must be considered and implemented into physicians and patient's information. To this purpose, the CAT decided to replace the proposed statements related to the Insertion site analysis by the applicant from section 5.1 to section 4.4 and to only mention the key messages of vector integration in liver cells and other body cells associated with a potential risk of malignancy beyond technical aspects (no preferential integration near genes of concern although some sites within 10 kb of proto-oncogenes and tumour suppressor genes). Moreover, a specific follow-up of 15 years was to be implemented for all ongoing/planned studies for the patients to be treated.

Safety in special populations

The data provided for ≥ 60-year-old patients are still too limited to draw any reliable conclusion on the safety profile for this subgroup of patients. The applicant has added in section 4.2 of the SmPC a statement including the information that data available in ≥ 65-year-old patients are limited. The applicant clarified also that the potential impact of age on the safety and efficacy profile will continue to be monitored in the post-approval setting (e.g., analyses of all SAEs, including malignancies, stratified by age), and any relevant findings will be summarised in periodic safety update reports, what is endorsed.

According to the applicant, Roctavian should not be used in women of childbearing potential, because the conditions for use in these patients have not yet been established. No female patients were included in the clinical development. No dedicated animal fertility/embryofoetal studies as well as no vector DNA ovarian shedding have been conducted with BMN 270 to establish an adequate waiting period after BMN 270 infusion following which female patients can become pregnant. There is neither information regarding the presence of BMN 270 in human milk, the effects on the breastfed infant, or the effects on milk production. As a matter of fact the indication also covers women (rare cases) but would currently be relevant to women without childbearing potential (subgroup of a rare subgroup). The applicant proposed to conduct a reproductive and developmental toxicity study in wild type CD-1 female mice, considered as an obligation to conduct post-authorisation measure (PAM), noted in the Annex II-D of the SmPC. The purpose of the study is to inform the impact of BMN 270 on embryo-fetal developmental toxicity in females of childbearing potential and establish an adequate waiting period after BMN 270 infusion following which female patients can become pregnant. Since the data on the draft synopsis is not sufficiently detailed, no further comment is raised.

As a precautionary measure, after administration of Roctavian, men must not donate semen for 6 months, and men and their female partners must prevent or postpone pregnancy for 6 months, as reflected in the SmPC. Additional Healthcare Professional Guide and Patient Alert Card provide these recommendations. The applicant justified the 6 months of contraception based data from two assays: iqPCR assay which confirms clearance of encapsidated (potentially infectious) vector DNA and qPCR assay which confirms clearance of all (residual) vector DNA forms in plasma and in semen. iqPCR and qPCR data have been updated. Therefore, the recommended duration of contraception (6 months) in the SmPC is based on the clearance time of encapsided (potentially infectious) vector DNA in semen by iqPCR assay (12 weeks) plus an additional 3 months to account for a complete spermatogenesis cycle

washout period and the time to first of the 3 consecutive BLOQ results of vector DNA by qPCR assay. The 6 months contraceptive measures are acceptable.

For 2 patients treated in 270-301, 35400 vg/mL and 24100 vg/mL in semen were observed beyond 6 months of contraception, at 25.9 weeks and at 31.8 weeks after BMN 270 administration respectively. A discussion about these cases of positive sample observed in 2 patients beyond 6 months of contraception was provided. The applicant provided the individual data of the clearance of encapsidated vector DNA in plasma and in semen assesses by iqPCR for these 2 patients. The values of the first of three consecutive BLD samples in plasma and in semen are consistent with those observed for all evaluated treated patients who cleared encapsidated (potentially infectious) vector DNA assessed by iqPCR in plasma and semen within 10.1 and 12.1 weeks of BMN 270 administration respectively.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Additional expert consultations

Given that this first gene therapy product in the field of haemophilia could shift the paradigm of the therapeutic management of patients with severe haemophilia A the CAT requested advice from an ad hoc expert group (AHEG) meeting to obtain experts (haematologists and hepatologists) and haemophilia patients representatives' views on how they would conciliate the uncertainties on long term data and the burden of corticosteroid with this gene therapy taking into account the current armamentarium of available medicines.

1. Based on the available data, do you think that this treatment will positively contribute to the current therapeutic armamentarium?

The group agreed unanimously that this treatment can be useful in the treatment of the disease in specific situations where benefits in terms of achieving stable sufficiently high factor VIII levels, avoidance of frequent factor VIII administration for at least a number of years outweigh the frequent burden and harms associated with immunosuppression and monitoring of liver function over a number of months, and the uncertainties about the long-term effects of the product. This complex balance of benefits and risks should be part of a conversation between doctors and patients, where available treatment options, evidence, uncertainties, patient objectives, and preferences are part of the decision. Key elements to be considered in such decisions should be identified and built into educational material or other formal risk minimisation measures.

Treatment decisions should include informed consent (including risks of gene therapy and the burdens and harms associated with management of hepatotoxicity) and follow a collaborative "shared decision making" approach ensuring that patients are optimally supported to consider available options, evidence and uncertainties, as well as their preferences, personal circumstances, and goals to make decisions that are right for them.

There is an opportunity to further optimise the utility of the treatment by identifying factors associated with hepatotoxicity and long-term effects, characterising the nature of the hepatotoxicity to guide patient selection and management of toxicity, and identifying factors associated with response. Despite the limitation of exploratory analyses in small subsets, this effort of data collection and analysis should continue in a systematic way. There is an opportunity to consider the contribution of registries at national and international level to optimise long-ter follow-up on efficacy and safety.

A clear and detailed monitoring programme and plan of what is going to be done if a significant adverse event is detected, e.g. cancer, should be in place.

2. How clinically relevant do you consider the current 2-year efficacy data and how do you value the current uncertainties with regards to the long-term effect?

Two-year efficacy data in terms of factor VIII levels and bleedings are considered sufficient to allow a discussion about the balance of benefits and risks that may be favourable for a subset of patients in the context of an informed decision (see above). The available longer term data are promising despite an apparent decrease of factor VIII levels over time but it is impossible to conclude at the moment about the long-term efficacy and safety of the product. The long-term effects should be further characterised.

3. What is your opinion on the ability to manage the risk for hepatotoxicity (early and long-term, due to patient related co-morbidity factors and induced by the product's risk for immunactivation and consequent need for steroid treatment) and the treatment burden associated with it?

The risk of hepatotoxicity was considered to be sufficiently managed with the propose protocol that was mainly based on empirical justifications. Therefore, there are important opportunities for better characterisation of the risk and optimise its management on the basis of evidence-based recommendations. Notwithstanding the need for optimisation, the risk is currently sufficiently characterised and managed to allow informed decisions. IN conclusion, there is a need to ensure that adequate evidence is generated to minimise the burden and side-effects of steroid treatment.

4. How do you rate the impact of the therapy on the patient's quality of life?

Therapy is expected to have a favourable effect on patients' quality of life at least in the short term and after hepatotoxicity has been successfully managed, and provided that treatment decisions are adequately informed and in line with patient preferences (see above). This effect is mainly based on the expected benefits of achieving stable sufficiently high factor VIII levels avoidance of frequent factor VIII administration in cases where this is considered important and outweighs the risks and uncertainties. There are also theoretical advantages of sustained factor VIII activity over time even if below the desired threshold.

However, it is difficult to conclude on the robustness of any claims of improved quality of life over existing treatments due to the lack of comparative data. Also, it is difficult to conclude on the robustness of the quality of life data presented from the studies due to the non-concurrent controlled design of the studies.

2.6.10. Conclusions on the clinical safety

Updated data submitted in Study 270-301 in 134 subjects showed that single IV infusion of BMN 270 was generally well tolerated in the majority of patients. All patients successfully completed their full-dose infusion of BMN 270, with no infusions requiring permanent termination prior to completion due to AEs. No patients discontinued from clinical trials as a result of an AE. Frequency of adverse events appears to decrease over time with no delayed adverse drug reactions. The main safety findings relate to transient ALT elevations (grade 1 to 3 in severity) observed in most patients that were treated with corticosteroids in most cases, or more rarely other alternative immune suppression reagents, to limit hepatocellular toxicity and possibly limit reduction of transgene expression over the period when ALT levels were elevated. A small number of patients experienced short-lived infusion reactions that were effectively mitigated by managing infusion rate and medications.

Nevertheless uncertainties on the clinical safety remain. Taking into account the different approaches in the use of corticosteroids across BMN 270 clinical trials prophylactic in study 270-201 and reactive in study 270-301 and the Rollover Population in 270-301 who received on the other side the longer

duration and higher total dose of corticosteroids (more than double the total dose of corticosteroids and for an average of 80 days longer), it is difficult to definitively determine if the applicant proposed corticosteroid regimen is the best corticosteroid regimen to achieve optimal long-term FVIII expression with the least AE given the variability in patient total corticosteroids exposure across the studies. Common, reversible, Grade 1/2 corticosteroid-related side effects have occurred with early and/or reactive use of corticosteroids. Isolated but more clinically important severe/serious corticosteroid-related side effects emerged with extended use of corticosteroids (higher total dose of corticosteroids over a longer total duration). It should be noted that due to changes in proposed strategies, the optimal CS regimen remains to be determined. To this purpose it is noteworthy that the applicant is still willing to test a prophylactic approach with the new study 270-303 and the ongoing studies 270-203 and 270-205. Results of these studies will need to be put into perspective with the data provided via the registry, where patients will receive an optimised reactive CS regimen and with the data provided from the pivotal study.

Uncertainty remains regarding the translation of the non-clinical finding of vector integration into a risk of tumorigenicity at the clinical level. Insertional mutagenesis by AAV vectors is identified as a potential risk and the applicant addresses it in line with the intended treated population by proposing additional measures and a much longer follow up monitoring (15 years).

Results of investigations on a case of acinic cell carcinoma of the parotid gland considered as unrelated by Biomarin and the independent DMC consulted were provided and confirmed the applicant's conclusions. The risk of rAAV integration contributing to tumorigenesis for this case therefore seems low with BMN 270, which is rather reassuring. Nevertheless, a small number of BMN 270 integrations were identified in each of the four parotid gland DNA samples. It is a lower BMN 270 vector quantities in the parotid gland compared to liver, as expected. Vector integration into human liver was clearly detected in all five liver biopsies available from 5 different subjects.

While the clinical relevance of Roctavian integration is unknown, a respective warning has been added to the SmPC and package leaflet to inform on the potential risk of malignancy as a result of vector integration in liver cells and in other body cells.

Overall, and having in mind the AHEG feed-back, it was considered that the following points should be considered when establishing the indication and timing of Roctavian use. The patients should be counseled as follows:

- No predictive factors for no or low responders have yet been identified. Patients who do not respond may still be exposed to long term risks.
- The experience of patients who have achieved five years follow-up is limited. The long term treatment effect cannot be predicted.
- There are no plans to re-administer the drug for patients who do not respond or have lost the response.
- Roctavian will, in most cases, require co-administration of corticosteroid treatment to manage the liver damage that this medicinal product induces.
- It should be ensured the availability of the patient for frequent monitoring of hepatic laboratory parameters and factor VIII activity after administration, as described in "Hepatic and factor VIII monitoring" in the SmPC.
- The patient's ability to receive corticosteroids that could be required for an extended time period should be evaluated. It should be ensured that the risks associated with the described regimen are likely to be acceptable for the individual patient. Experience with regimens using other immunosuppressive agents is limited (as reflected in SmPC see section 4.8).
- The patient's existing medications prior to administration of Roctavian should be reviewed to determine if they should be modified to:

- prevent anticipated drug interactions with corticosteroids (as reflected in the SmPC section 4.5) and
- minimise the potential for reduced therapeutic effect of Roctavian or adverse effects (e.g., limiting the use of hepatotoxic agents, including alcohol, or prothrombotic medications

- There is a potential risk of malignancy in relation to vector genome integration after treatment with Roctavian. Although no cases have been reported so far, a long term surveillance of 15 years is to be recommended for patients.

These aspects are covered in the SmPC and Package leaflet and in the educational materials in the RMP, as reflected in Annex II.

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

2.7. Risk Management Plan

2.7.1. Safety concerns

Summary of Safety Concerns	
Important identified risks	• Hepatotoxicity • Infusion reactions (including hypersensitivity)
Important potential risks	• Thromboembolic events • Development of FVIII inhibitors • Transmission to third parties (horizontal transmission) • Germline transmission • Risk of malignancy in relation to vector integration in the DNA of body cells
Important missing information	• Long-term effect • Use in patients with liver impairment • Use in female patients

2.7.2. Pharmacovigilance plan

Safety studies:

Study Status	**Summary of Objectives**	**Safety Concerns Addressed**	**Milestones**	**Due Dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
None imposed for safety reasons.				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
No Specific Obligation imposed for safety reasons.				

Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Category 3 - Required additional pharmacovigilance activities				
270-201 A Phase 1/2, Dose-Escalation, Safety, Tolerability and Efficacy Study of BMN 270, an Adenovirus-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII in Patients with Severe Haemophilia A Phase 1/2 Ongoing (fully enrolled)	The primary objectives of the study are: To assess the safety of a single intravenous administration of a recombinant AAV5 encoding human coagulation FVIII (AAV5-hFVIII-SQ) vector. To determine the dose of AAV5-hFVIII-SQ required to achieve FVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated FVIII activity in individuals with haemophilia A will be determined and correlated to an appropriate BMN 270 dose. The secondary objectives of the study are: To describe the immune response to the FVIII transgene and AAV capsid proteins following systemic administration of AAV5-hFVIII-SQ To assess the impact of BMN 270 on the frequency of FVIII replacement therapy during the study To assess the impact of BMN 270 on the number of bleeding episodes requiring treatment during the study	Hepatotoxicity Infusion reactions Thromboembolic events Development of FVIII inhibitors Transmission to third parties Germline transmission Risk of malignancy in relation to vector integration in the DNA of body cells Long-term effect	Date of original protocol: Date first posted to ClinicalTrials.gov: Start date of data collection: Interim Reports: Interim CSR for initial MAA Interim CSR for new MAA Last Patient Out: Final Report:	10 February 2015 15 October 2015 29 July 2015 25 October 2019 9 April 2021 Anticipated 30 June 2024 Anticipated 31 December 2024
270-302 A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII at a dose of 4E13 vg/kg in Haemophilia A Patients with Residual FVIII Levels ≤ 1 IU/dL Receiving Prophylactic FVIII Infusions Phase 3 Ongoing (not currently enrolling)	The safety objectives of the study are to: Evaluate the safety of BMN 270.	Hepatotoxicity Infusion reactions Thromboembolic events Development of FVIII inhibitors Transmission to third parties Germline transmission Risk of malignancy in relation to vector integration in the DNA of body cells Long-term effect	Date of original protocol: Date first posted to ClinicalTrials.gov: Start date of data collection: Interim Reports: Interim CSR for initial MAA Interim CSR for new MAA Last Patient Out:	15 September 2017 8 January 2018 23 May 2018 19 August 2019 30 April 2021 Anticipated 30 June 2023

Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
			Final Report:	Anticipated 31 December 2023
Healthcare professional Survey (Planned – study number not yet assigned)	To evaluate effectiveness of educational material provided as additional risk minimisation measures	Hepatotoxicity Thromboembolic events Transmission to third parties Germline transmission	Draft synopsis.	June 2021
			Registration in EU PAS Register	31 December 2022
			Final protocol to be submitted	31 March 2023
			Survey Conducted:1, 3, and 5 years post-launch in first European country	31 December 2023 31 December 2025 31 December 2027
			Interim and Final Survey Reports: (in accordance with Marketing Authorisation commitments) Interim: Final:	30 June 2024 30 June 2026 30 June 2028
			Publication (anticipated within 2 years of final report)	30 June 2030
Study Summary: Embryo-Foetal Developmental Toxicity Study	To inform the impact of BMN 270 on fertility, general toxicity, teratology, and germline transmission in females of childbearing potential and establish an adequate waiting period after BMN 270 infusion following which female patients can become pregnant	Use in female patients	Draft synopsis (with RMP) Protocol submission Final report	February 2022 15 February 2023 31 December 2023

Studies imposed primarily for efficacy reasons that will also provide safety results:

Study Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Date
Efficacy studies which are conditions of the marketing authorisation				
270-601 A Non-Interventional, Multi-National, Longitudinal Study of Patients Treated with Roctavian (valoctocogen	This study is being undertaken to better characterize the long-term effectiveness and safety of Roctavian in patients in a real-world setting. The study aims to assess the long-term effectiveness of the product in a broader population to further inform the risk-benefit balance of Roctavian and to provide information on the long-term impact of treatment with Roctavian. In addition, the study	To describe treatment response including the bleeding profile and long-term durability of FVIII expression, along with quality of life in patients post	Protocol Submission: Draft with RMP; Final protocol	June 2021 31 March 2023
			Registration in EU PAS Register	31 December 2022
			Start of Data Collection:	30 June 2023
			Annual Status Reports:	31 December 2023 31 December 2024

Study Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Date
e roxaparvovec) Planned	aims to assess the frequency and incidence rate of safety events identified in the Pharmacovigilance and Risk Management Plan including hepatoxicity, thromboembolic events, infusion reactions (including hypersensitivity), malignancies, and development of FVIII inhibitors. **Primary Objectives:** • To describe the bleeding profile and long-term durability of FVIII expression in patients administered Roctavian • To describe the use of exogenous factor and non-factor replacement treatment(s) in patients administered Roctavian • To describe the change in clinical outcome assessments (ie, Haemophilia-specific quality of life questionnaire [Haemo-QoL-A], EuroQol 5 Dimension 5 Level instrument [EQ-5D-5L], and Work Productivity and Activity Impairment plus Classroom Impairment Questions: Hemophilia Specific [WPAI+CIQ:HS]) in patients administered Roctavian **Secondary Objectives:** • To quantify and characterize the risk of SAEs and suspected ADRs in patients administered Roctavian • To quantify and characterise the risk of targeted adverse events (TAEs) of hepatotoxicity, thromboembolic events, infusion reactions, new malignancies, and development of FVIII inhibitors in patients with haemophilia A administered Roctavian	administration of Roctavian. Effectiveness endpoints will include: ABR for treated bleeds and percentage of patients with zero (0) bleeds, stratified according to time from dose administration Change over time of FVIII levels Use of exogenous factor and non-factor replacement treatment Change over time in patient reported quality of life		31 December 2025 31 December 2026 31 December 2027 31 December 2028 31 December 2029 31 December 2030 31 December 2031 31 December 2032 31 December 2033 31 December 2034 31 December 2035 31 December 2036 31 December 2037 31 December 2038 31 December 2039 31 December 2040 31 December 2041
			Interim Report:	30 June 2029 30 June 2034 30 June 2039
			End of Data Collection: assuming approximately 3-year recruitment period	30 September 2041
			Final Report: assuming approximately 3-year recruitment period	30 September 2042
270-801 A Retrospective	This study is being undertaken to better characterize the long-term safety and outcomes of patients treated with Roctavian in a real-	To describe treatment response including the	Protocol Submission: Draft with RMP Final protocol	February 2022 31 March 2023

Study Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Date
Cohort Study of Patients Treated with Roctavian (valoctocogene roxaparvovec) : An Analysis of Patient Registries Planned	world setting based on the safety profile outlined in the Pharmacovigilance and Risk Management Plan. **Primary objective**: • To quantify and characterize the risk of targeted adverse events (TAEs) of hepatoxicity, thrombotic events, infusion reactions, new malignancies, and development of FVIII inhibitors among patients with HA administered Roctavian. **Secondary objectives**: • To quantify and characterize the risk of suspected adverse drug reactions (ADRs) in patients administered Roctavian. • To describe the bleeding profile and long-term durability of FVIII expression in patients administered Roctavian. • To describe the use of exogenous factor and non-factor replacement treatment(s) in patients administered Roctavian. • To describe changes in quality of life, as measured by the Euro-QoL Health Status Assessment: 5 Dimensions, 5 Levels of Severity (EQ-5D-5L), in patients administered Roctavian. • To quantify and characterize the risk of TAEs among patients with HA treated with haemostatic treatments, stratified by disease severity and treatment regimen.	bleeding profile and long-term durability of FVIII expression, in patients post administration of Roctavian. Effectiveness endpoints will include: ABR for treated bleeds and percentage of patients with zero (0) bleeds, stratified according to time from dose administration Change over time of FVIII levels Use of exogenous factor and non-factor replacement treatment	Start of Data Collection:	30 June 2023
			Annual Status Reports:	31 December 2023 31 December 2024 31 December 2025 31 December 2026 31 December 2027 31 December 2028 31 December 2029 31 December 2030 31 December 2031 31 December 2032 31 December 2033 31 December 2034 31 December 2035 31 December 2036 31 December 2037 31 December 2038 31 December 2039 31 December 2040 31 December 2041 31 December 2042 31 December 2043
			Interim Report:	30 June 2029 30 June 2034 30 June 2039
			End of Data Collection: Contingent on last data extract available from data sources for 10th year of follow-up for 720 patients	Anticipated 30 September 2043
			Final Report: assuming ~5 years to reach 720 patients enrolled in WFHGTR)	Anticipated 30 June 2044
270-401 (Planned) A Long-Term Follow-Up Study in Subjects with Haemophilia A Who Received BMN 270, an Adeno-	The purpose of this study is to monitor the safety and efficacy of BMN 270 long-term in subjects who received the drug in a clinical study. Subjects will be enrolled in 270-401 following completion of 5 years in the dosing study and will be followed for approximately 10 years in the	To assess long-term effectiveness of BMN 270	Final Protocol	13 April 2022
			Start date of data collection	Anticipated 28 September 2022
			Interim Reports	None planned
			End of data collection	TBD
			Final Report	31 July 2038

Study Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Date
Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII in a Prior BioMarin Clinical Trial	long-term study (15 years total from BMN 270 dosing).			
Efficacy studies which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
270-303 A Phase 3b, Single Arm, Open-Label Study to Evaluate the Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII, with Prophylactic Corticosteroids in Haemophilia A Patients Ongoing	The primary efficacy objective of the study is to: • Assess the efficacy of BMN 270 with prophylactic corticosteroids defined as FVIII activity, as measured by chromogenic substrate assay, during Weeks 49-52, following intravenous infusion of BMN 270 The secondary efficacy objectives of the study are to: • Assess the impact of BMN 270 with prophylactic corticosteroids on the use of exogenous FVIII replacement therapy from Week 5 to last visit by data cutoff (for Week 52 analysis) for subjects receiving prior FVIII prophylaxis or on use of emicizumab from Week 27 to last visit by data cutoff (for Week 52 analysis) for subjects receiving prior emicizumab prophylaxis • Assess the impact of BMN 270 with prophylactic corticosteroids on the number of bleeding episodes requiring exogenous FVIII replacement therapy from Week 5 to last visit by data cutoff (for Week 52 analysis) for subjects receiving prior FVIII prophylaxis or on use of emicizumab from Week 27 to last visit by data cutoff (for Week 52 analysis) for subjects receiving prior emicizumab prophylaxis	To further substantiate the durability of the efficacy of BMN 270 in the context of prophylactic steroids.	Date of original protocol:	28 February 2020
			Date first posted to ClinicalTrials.gov:	26 March 2020
			Start date of data collection:	8 December 2020
			Interim Reports:	30 September 2023
			End of data collection	Anticipated 31 January 2027
			Final Report:	Anticipated 30 September 2027

Study Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Date
	• Assess the impact of BMN 270 with prophylactic corticosteroids on quality of life as measured by the Haemo-QoL-A questionnaire at Week 52 of the study compared to baseline The safety objectives of the study are to: • Evaluate the short-term safety of BMN 270 with prophylactic corticosteroids following intravenous infusion of BMN 270 • Assess the long-term safety of BMN 270 with prophylactic corticosteroids			
270-301 A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII in Haemophilia A Patients with Residual FVIII Levels ≤ 1 IU/dL Receiving Prophylactic FVIII Infusions Phase 3 Ongoing (fully enrolled)	• The primary efficacy objective of the study is to: • Assess the efficacy of BMN 270. The secondary efficacy objectives of the study are to: • Assess the impact of BMN 270 on usage of exogenous FVIII replacement • Assess the impact of BMN 270 on the number of bleeding episodes requiring exogenous FVIII replacement therapy. The safety objectives of the study are to: • Evaluate the safety of BMN 270. • Assess the long-term safety of BMN 270.	To further substantiate the durability of the efficacy of BMN 270 and to enrich the pharmacokinetic model to achieve reliable prediction beyond 5 years.	Date of original protocol:	14 August 2017
			Date first posted to ClinicalTrials.gov:	13 December 2017
			Start date of data collection:	19 December 2017
			Interim Reports: Interim CSR for initial MAA Interim CSR (52-Week Analysis) for new MAA Additional interim CSR	25 October 2019 27 May 2021 Planned 30 December 2022
			End of data collection:	Anticipated November 2024
			Final Report:	Anticipated 30 June 2025
270-205 A Phase 1/2 Safety, Tolerability, and Efficacy Study of BMN	Study 270-205 is a Phase 1/2, two-part, open-label study in HA patients with FVIII activity ≤ 1 IU/dL who have developed FVIII neutralizing antibodies (inhibitors) during HA treatment. Subjects eligible for this study	To assess efficacy of BMN 270 in patients with active or prior inhibitors	Date of original protocol	28 February 2020
			Date first posted to ClinicalTrials.gov:	28 December 2020

Study Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Date
270, an Adeno-Associated Virus Vector-Mediated Gene Transfer of Human Factor VIII in Haemophilia A Patients with Active or Prior Inhibitors Phase 1/2 Ongoing	must have either documented evidence of no immunological tolerance to exogenous FVIII (Part A), or demonstrated immunological tolerance to FVIII and a negative inhibitor test (<0.6 BU per cNBA) per central lab at Screening (Part B). The primary efficacy objective of the study is to: • Assess the safety of a single IV administration of BMN 270 in HA subjects with active inhibitors (Part A), or prior inhibitors (Part B) The secondary efficacy objectives of the study are to: • Assess the efficacy of BMN 270 as measured by FVIII activity together with the level of inhibitor titre (Part A) and the recurrence of inhibitors (Part B). • Assess the impact of BMN 270 on the use of haemophilia therapy. • Assess the impact of BMN 270 on the number of bleeding episodes requiring pharmacologic intervention • Assess the impact of BMN 270 on quality of life as measured by the Haemo-QoL-A questionnaire The tertiary efficacy objectives of the study are to: • Assess the impact of BMN 270 on quality of life as measured by additional patient-reported outcome (PRO) instruments • Assess the safety and efficacy of emicizumab transition to BMN 270		Start date of data collection:	22 November 2021
			Interim CSR:	30 September 2023
			End of data collection:	Anticipated 15 December 2027
			Final Report:	Anticipated 31 December 2028
270-203 A Phase 1/2 Safety, Tolerability, and Efficacy Study of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer	The primary objective of the study is to: • Assess the safety of a single intravenous administration of BMN 270 in severe HA subjects with pre-existing antibody to AAV5 vector capsid, including development of FVIII neutralizing antibody	To assess efficacy of BMN 270 in patients with pre-existing AAV5 antibodies	Date of original protocol:	29 September 2017
			Date first posted to ClinicalTrials.gov:	11 May 2018
			Start date of data collection:	24 April 2018

Study Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Date
of Human Factor VIII in Haemophilia A Patients with Residual FVIII Levels ≤ 1 IU/dL and Preexisting Antibodies Against AAV5 Phase 1/2 Ongoing (enrolling)	Secondary objectives of the study are to: • Assess the efficacy of BMN 270 at Week 26 • Assess the impact of BMN 270 on usage of exogenous FVIII replacement therapy • Assess the impact of BMN 270 on the number of bleeding episodes requiring exogenous FVIII therapy • Evaluate the pharmacodynamics of FVIII expression following IV infusion of BMN 270 • Assess the impact of BMN 270 on patient-reported outcomes (PROs)		Interim Reports: Interim CSR for initial MAA Interim CSR for new MAA Additional Interim CSR	17 July 2019 22 April 2021 30 September 2023
			End of data collection:	Anticipated 30 June 2027
			Final Report:	Anticipated 31 December 2027

2.7.3. Risk minimisation measures

Safety Concern	Risk minimisation measures	Pharmacovigilance Activities
Hepatotoxicity	Routine risk minimisation measures: **SmPC** Section 4.3 Section 4.4 Section 4.8 **PL** Section 2 Section 3 Section 4 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. Additional risk minimisation measures: Healthcare Professional Guide Patient Guide Patient Card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Hepatic Reactions Directed Follow-up Report Form (DFRF) Additional pharmacovigilance activities: 270-601 (final study report 30 September 2042) 270-801 (final study report 30 June 2044) 270-201 (final study report 31 December 2024) 270-203 (final study report 31 December 2027) 270-205 (final study report 31 December 2028) 270-301 (final study report 30 June 2025) 270-302 (final study report 31 December 2023) 270-303 (final study report 30 September 2027) 270-401 (final study report 31 July 2038) Healthcare professional survey (final study report 30 June 2028)

Infusion reactions (including hypersensitivity)	Routine risk minimisation measures: **SmPC** Section 4.2 Section 4.3 Section 4.4 Section 4.8 **PL** Section 2 Section 4 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: 270-601 (final study report 30 September 2042) 270-801 (final study report 30 June 2044) 270-201 (final study report 31 December 2024) 270-203 (final study report 31 December 2027) 270-205 (final study report 31 December 2028) 270-301 (final study report 30 June 2025) 270-302 (final study report 31 December 2023) 270-303 (final study report 30 September 2027)
Thromboembolic events	Routine risk minimisation measures: **SmPC** Section 4.4 **PL** Section 2 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. Additional risk minimisation measures: Healthcare Professional Guide Patient Guide Patient Card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: 270-601 (final study report 30 September 2042) 270-801 (final study report 30 June 2044) 270-201 (final study report 31 December 2024) 270-203 (final study report 31 December 2027) 270-205 (final study report 31 December 2028) 270-301 (final study report 30 June 2025) 270-302 (final study report 31 December 2023) 270-303 (final study report 30 September 2027) 270-401 (final study report 31 July 2038) Healthcare professional survey (final study report 30 June 2028)
Development of FVIII inhibitors	Routine risk minimisation measures: **SmPC** Section 4.4 Section 4.8 **PL** Section 2 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: 270-601 (final study report 30 September 2042) 270-801 (final study report 30 June 2044) 270-201 (final study report 31 December 2024)

	be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. Additional risk minimisation measures: Healthcare professional guide Patient Guide Patient Card	270-203 (final study report 31 December 2027) 270-205 (final study report 31 December 2028) 270-301 (final study report 30 June 2025) 270-302 (final study report 31 December 2023) 270-303 (final study report 30 September 2027) 270-401 (final study report 31 July 2038) Healthcare professional survey (final study report 30 June 2028)
Transmission to third parties (horizontal transmission)	Routine risk minimisation measures: **SmPC** Section 4.4 Section 5.2 **PL** Section 2 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. Additional risk minimisation measures: Healthcare Professional Guide Patient Guide Patient Card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: 270-201 (final study report 31 December 2024) 270-203 (final study report 31 December 2027) 270-205 (final study report 31 December 2028) 270-301 (final study report 30 June 2025) 270-302 (final study report 31 December 2023) 270-303 (final study report 30 September 2027) 270-401 (final study report 31 July 2038) Healthcare professional survey (final study report 30 June 2028)
Germline transmission	Routine risk minimisation measures: **SmPC** Section 4.4 Section 4.6 Section 5.2 **PL** Section 2 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. Additional risk minimisation measures: Healthcare Professional Guide Patient Guide Patient Card	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: 270-201 (final study report 31 December 2024) 270-203 (final study report 31 December 2027) 270-205 (final study report 31 December 2028) 270-301 (final study report 30 June 2025) 270-302 (final study report 31 December 2023) 270-303 (final study report 30 September 2027) Healthcare professional survey (final study report 30 June 2028)
Risk of malignancy in relation to	Routine risk minimisation measures: **SmPC**	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:

vector integration in the DNA of body cells	Section 4.4 Section 5.3 **PL** Section 2 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. Additional risk minimisation measures: Healthcare Professional Guide Patient Guide Patient Card	None Additional pharmacovigilance activities: 270-601 (final study report 30 September 2042) 270-801 (final study report 30 June 2044) 270-201 (final study report 31 December 2024) 270-203 (final study report 31 December 2027) 270-205 (final study report 31 December 2028) 270-301 (final study report 30 June 2025) 270-302 (final study report 31 December 2023) 270-303 (final study report 30 September 2027) 270-401 (final study report 31 July 2038) Healthcare professional survey (final study report 30 June 2028)
Long-term effect	Routine risk minimisation measures: **SmPC** Section 4.4 **PL** Section 2 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. Additional risk minimisation measures: Healthcare Professional Guide Patient Guide	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: 270-601 (final study report 30 September 2042) 270-801 (final study report 30 June 2044) 270-201 (final study report 31 December 2024) 270-203 (final study report 31 December 2027) 270-205 (final study report 31 December 2028) 270-301 (final study report 30 June 2025) 270-302 (final study report 31 December 2023) 270-303 (final study report 30 September 2027) 270-401 (final study report 31 July 2038) Healthcare professional survey (final study report 30 June 2028)
Use in patients with liver impairment	Routine risk minimisation measures: **SmPC** Section 4.2 Section 4.4 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: None

Use in female patients	Routine risk minimisation measures: **SmPC** Section 4.6 **PL** Section 2 Other routine risk minimisation measures beyond the Product Information: **Legal Status:** Medicinal product subject to restricted medical prescription. Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: Embryo-Foetal developmental toxicity study

2.7.4. Conclusion

The CAT considers that the risk management plan version 1.0 is acceptable.

The CHMP endorses the CAT conclusion on the RMP as described above.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

A request for a translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant proposing that the details listed in Article 54 appear in only one official language (English) on all packaging components (vial and outer carton) and the package leaflet. The main ground of the justification was the low estimated number of patients treated per country due to the low incidence/prevalence of the condition in the EU and the fact that the medicinal product will

administered by healthcare professional in a clinical setting.

The QRD Group partially accepted the translation exemption for the use of English only labels on the immediate packaging (vial), but the Group requested that the outer carton have dual language English(EN)/Germany(DE) labelling. On the other hand, no consensus was agreed regarding the acceptability of an EN package leaflet, therefore, the MAH should contact the Member States with a translation exemption request linked to Article 63(3).

2.9.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Roctavian (valoctocogene roxaparvovec) is included in the additional monitoring list as

- It contains a new active substance
- It is a biological product
- It has a PASS imposed
- It is approved under a conditional marketing authorisation

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Roctavian is for treatment of severe haemophilia A (HA; congenital factor VIII deficiency) in adult patients without a history of factor VIII inhibitors and without detectable antibodies to adeno associated virus serotype 5 (AAV5).

3.1.2. Available therapies and unmet medical need

Factor VIII prophylaxis:

The current standard of care for HA in developed countries has been prophylactic infusion of exogenous FVIII two to three times per week, or episodically (also referred to as on-demand) at the time of a bleeding event (Srivastava 2020). Treatment with existing FVIII replacement products also requires frequent injections, which are associated with complications and a negative impact on HRQoL. The goals of prophylaxis with FVIII are to increase trough FVIII activity to at least a moderate level (1–5 IU/dL) and hence reduce the occurrence of bleeding episodes and subsequent joint damage (Manco-Johnson 2007). Extended half-life (EHL) FVIII products have been engineered to reduce the clearance rate of exogenously administered FVIII (e.g., by developing FVIII-Fc fusion proteins or via conjugation to polyethylene glycol), thereby increasing their half-lives to 18-19 hours, compared to 12-18 hours for conventional FVIII products. This half-life extension permits both a reduction in the number and frequency of injections required to achieve a similar degree of clinical efficacy compared to standard half-life FVIII concentrates (Berntorp, 2016).

Emicizumab:

Emicizumab is a humanised, bispecific monoclonal antibody that binds to both activated factor IX and factor X, thereby mimicking the function of activated FVIII. It was recently approved for the treatment of person with HA with or without FVIII inhibitors. Emicizumab represents an incremental improvement in therapeutic burden over replacement FVIII products by reducing the treatment administrations (administration every 1-4 weeks).

There is currently no curative treatment for HA.

Despite advancements in the management of HA, there remains an unmet medical need since available treatment options, including non-FVIII replacement therapies (ie, emicizumab), all require long-term, chronic treatments with a high degree of compliance to the prescribed treatment schedule to be effective.

3.1.3. Main clinical studies

The main evidence of Roctavian came from the dose-response study 270-201 and the pivotal study 270-301.

Study 270-201 was a first-in-human, phase I/II, dose escalation study with sequential enrolment. Based on the exploratory dose-finding study 270-201, the 6E13 vg/kg dose has been chosen for the confirmatory trial.

The pivotal Study 270-301, a Phase 3 single-arm, non-randomised, open-label study in haemophilia A patients with residual FVIII levels ≤ 1 IU/dL treated continuously with prophylactic exogenous FVIII for a minimum of one year prior to enrolment (N = 134). In this study adult patients are treated at $6x10^{13}$ vg/kg.

Study 270-301 included male subjects ≥ 18 years of age with severe haemophilia A (residual FVIII levels ≤ 1 IU/dL), without history of FVIII inhibitor and without detectable pre-existing antibodies to the AAV5 capsid.

One hundred twelve (112) patients previously participated in a non-interventional study with at least 6 months of prospectively collected baseline data prior to enrolment in study 270-301. One hundred six of the 134 patients initiated corticosteroid treatment only in response to ALT elevation (generally starting at 60 mg/day and gradual tapering thereafter

The primary efficacy endpoint was change in factor VIII activity at week 104 post Roctavian infusion from baseline (imputed as 1 IU/dL), as measured by chromogenic substrate assay (CSA). The secondary efficacy endpoints were change from baseline in ABR requiring exogenous factor VIII and annualised use of exogenous factor VIII in the post factor VIII prophylaxis period.

Updated efficacy data for the proposed dose are available from 134 subjects from the pivotal trial 270-301 at Week 104 and from the 7 subjects in cohort 3 of the supportive trial 270-201 at Week 260.

3.2. Favourable effects

In the pivotal phase 3 study 270-301, 101 of 134 (75.4%) subjects had a median FVIII activity level ⩾ 5 IU/dL at Week 104. The median FVIII activity level (chromogenic assay) for the ITT Population was 23.92 IU/dL at Week 49-52 and 11.75 IU/dL at Week 104, corresponding to FVIII level of mild haemophilia (5-<40 IU/dL). A total of 33 of the 134 (24.6%) subjects in study 270-301 were considered low-responders, achieving a median FVIII activity level <5 IU/dL at Weeks 104 (12% at Week 49-52).

In the supportive phase I/II study, 5 of 7 subjects (cohort 3) developed substantially higher levels of FVIII activity at Week 260. The mean (median) FVIII activity for cohort 3 is 64.3 (60.3) at year 1, 36.4

(26.2) at year 2, 32.7 (19.9) at year 3,24.2 (16.4) at year 4 and 11.6 (8.2) IU/dL, showing a sustained, although decreasing, production of FVIII. Two of 7 subjects were considered low-responders, one reached a level of 7.9 IU/dL at week 16 which has declined to 3.1 IU/dL at week 208 and one reached a peak of 77.4 IU/dL at Week 26 then progressively declined until 0 IU/dL at Week 208.

This translates into a clinically meaningful decrease in ABR post-infusion. The Rollover Population, who has the highest quality baseline data available, showed a reduction of ABR of 84.5% over the efficacy evaluation period. 83.9% of subjects in the Rollover population had no treated bleeds from Week 5 or beyond post-infusion in study 270-301. At 5 years of follow-up in study 270-201, 5 out of 7 (71.4%) subjects in the 6E13 vg/kg dosing cohort had no bleeding episodes requiring FVIII treatment from Week 5. In low responder patients (2 in the 6E13 vg/kg cohort of study 270-201 and 33 in the ITT population of study 270-301), while bleeding risk is not abolished, ABR was nevertheless reduced when compared to their pre-infusion rate in 94% (31/33) of subjects in study -301 at Week 52.

A drastic reduction in FVIII infusion is also observed in all patients who received the 6E13 vg/kg dose in study -201 or -301. External factor VIII consumption was reduced by 98.2% from a mean (SD) of 3961.17 (1751.47; median 3754.42) IU/kg/year to a mean (SD) of 69.90 (209.22; median 0.00) IU/kg/year. Of the 112 Rollover Population subjects, 82 (73.2%) required no exogenous FVIII use from the end of the FVIII prophylaxis period to Year 2 and 68 (60.7%) over the entire efficacy evaluation period. 6 subjects returned to continuous prophylaxis posttreatment (5 with FVIII replacement therapy, 1 with emicizumab), with both individuals experiencing a satisfactory treatment response following resumption of prophylaxis.

Even in patients in whom FVIII activity levels did not reach expected levels, a reduction in annualised FVIII usage is observed, i.e. reduction of annualised FVIII utilisation by ≥90% in 75% (12/16) of the low-responders in study -301. Most subjects in both 270-201 (3/7 in the 6E13 cohort) and the Rollover population of 270-301 (75/112) had no FVIII utilisation up to the data cut-off. The main beneficial effect of Roctavian appears to be the reduction of utilisation/infusion of exogenous FVIII, i.e. a reduction from baseline to Week 5 to DCO of 98.2% in -301 and 96.3% in -201.

3.3. Uncertainties and limitations about favourable effects

Methodological issues

- The pivotal clinical evidence for this product comes from uncontrolled, non-randomised, open-label, studies only. Consequently, internal and external validity of statistical conclusions hinges on a large number of untestable assumptions. Bias due to selection cannot be excluded.

- In the context of a non-randomised single arm open-label pivotal study, several important changes were made to the study design and statistical methods compared to the original protocol. Updates were made as part of several protocol amendments and SAP versions, with impacts on the sample size, IAs, multiplicity adjustment procedure and primary / secondary analysis definitions. Due to the content and timing of these updates, it is not possible to consider the study type I error as being formally controlled across primary and secondary endpoints. Therefore, while a therapeutic benefit is acknowledged in view of the FVIII increased activity in patients with severe haemophilia A having stopped FVIII before enrolling in the study, claim of statistical significance were requested as being removed, which was accepted by the applicant.

Variability of median FVIII levels

A wide variability was observed in the level of FVIII activity (median FVIII levels by CS assay at W49-52) achieved by the patients receiving the 6E13 vg/kg dose in each study, ranging from 12.5-126.6

IU/dl in 270-201 and from 0.0, 231.2 in the ITT population of study 270-301. The applicant seems to have investigated multiple sources of variability (including baseline or demographic characteristics, duration of study drug administration, concomitant medication use, PD parameters and manufacturing-related parameters); a trend of lower FVIII activity levels at week 104 was observed in Black or African-American subjects within the study population. Subjects with intron 22 inversion appeared to have higher FVIII activity than those without. These identified factors of variability remained inconclusive trends based on small subgroup numbers, lack of consistency with the analyses performed at one year and the nature of the analyses (post-hoc exploratory analyses) with limited reliability. The applicant has committed to provide further updated analyses on potential factors that impact interpatient FVIII activity level variability with data derived from ongoing studies 270-203, -205 and 303 to identify predictive factors of no or low responders.

Low-responders

In the phase 3 study, a total of 33 of the 134 (24.6%) subjects were no or low-responders, achieving a median FVIII activity level <5 IU/dL at Weeks 104.

Of the 33/134 subjects in Study 270-301 who achieved a median FVIII activity level <5 IU/dL at Weeks 104, five subjects restarted continuous FVIII prophylaxis two years after Roctavian infusion (one started emicizumab, the others resumed continuous FVIII prophylaxis). Three out of 33 low-responders had ABR >1 and a higher ABR post BMN 270 infusion compared to baseline.

As a consequence, the reason behind some (even though few) patients not reaching adequate levels of FVIII activity following infusion of Roctavian remains unknown at this stage and treatment failure cannot be predicted for future patients. As Roctavian transduces liver cells, the treatment cannot be interrupted or stopped in case of insufficient efficacy response, and the patient has to bear the potentially life-long burden of adverse outcomes without a clinically relevant improvement of disease control. The applicant has committed to provide further updated analyses on potential factors that impact interpatient FVIII activity level variability with data derived from ongoing studies 270-203, -205 and 303 to identify predictive factors of no or low responders as part of the Specific Obligations of the CMA.

FVIII Activity, ALT Elevations, and Corticosteroid Use

Rise in ALT is an expected adverse event of AAV-based gene therapies (immune toxicity against AAV capsid), which although generally asymptomatic, can be associated with loss or reduction of transgene expression as a likely consequence of loss of transduced hepatocyte. Management of ALT elevation by oral corticosteroids changed several times during the conduct of the supportive studies, making comparison of study results more complex. In study 270-201, 4 of the 7 subjects in the 6E13 cohort received prophylactic course of corticosteroids starting at week 3 per the protocol, while 3 subjects were initiated corticosteroids earlier than week 3 in response to ALT elevation.".Patients in study 270-301 received therapeutic corticosteroids with threshold that have been amended during the study. While in 270-201, patients received corticosteroids after a mean time of 3.0 weeks after Roctavian administration, this duration was 9.9 weeks in 270-301. In the context of the conditional marketing authorisation, additional information will be collected from the imposed studies as specific obligations to determine the adequate corticosteroid regimen that could mitigate ALT increase and preserve FVIII activity while minimising AEs related to corticotherapy.

Persistence of the effect

Evidence for a sustained FVIII activity clinically meaningful is based on two-year follow-up in the pivotal 270-301 study (N=134) and five-years follow-up in the supportive 270-201 (N=7). The evaluation of long-term efficacy depends exclusively on the outcomes of the 7 subjects from 270-201 and is therefore not considered to be sufficient. An observation period of 104 weeks in 270-301 is

considered not sufficient to estimate the sustainability of FVIII activity with a satisfactory degree of certainty.

The gradual decline of FVIII activity observed further to a peak at approximately 6 months raised uncertainties on the durability of the treatment effect. It is unknown if the FVIII activity decrease might be sufficient to support hemostatic efficacy for multiple years or decline below the threshold of 5 IU/dL.

Moreover the durability of treatment effect cannot rely on the presence of stable, circular episomes of the FVIII transgene as it seems not correlated to FVIII activity according to the provided liver biopsies (N=5) from Study 270-201. Dose-dependent increase in circular full-length and ITR-Fused BMN 270 genomes were detected 2.6-4.1 years following BMN 270 dosing in the 5 subjects. One subject treated at 4E13 vg/kg was however FVIII non-responder with a level of hFVIII-SQ RNA ~ 10-fold lower than the second subject treated at 4E13 vg/kg while the four other participants showed dose-dependent FVIII-SQ RNA levels. Data suggest that presence of circular BMN 270 vector genome in hepatocytes is likely not predictable of long-term FVIII-SQ RNA and plasma FVIII activity. Those investigations will nevertheless be pursued with liver biopsies from patients in study 270-301.

As requested, the applicant has developed a model to predict durability beyond the 2 years data from study 270-301. Insufficient level of information is currently available to substantiate the prediction made and updated data will have to be provided though the response to the SOB requiring the 5 years follow up of the pivotal study. In any case the model will have to be enriched with the longer term data from study 270-301 to enhance the reliability of the prediction. In the context of the conditional marketing authorisation, 5 years follow up data from the pivotal study 270-301 will have to be provided.

3.4. Unfavourable effects

The analysis of the safety profile of Roctavian relies on 151 patients who received Roctavian infusion, among 4 ongoing clinical studies, of which 141 received Roctavian at the dose of 6E13 vg/kg (Label Population).

All patients experienced at least 1 TEAE. A majority of patients experienced only Grade 1 (mild) or Grade 2 (moderate) TEAEs. Grade 3 events occurred in approximatively 30% of patients. 2 patients reported 5 Grade 4 events and 1 Grade 5 event, all assessed as unrelated to Roctavian.

Hepatotoxicity: Elevations in ALT meeting the EOSI definition were reported in 133 patients (88.1%) (119 patients from 270-301) following Roctavian administration. No patient experienced a Grade 4 or Grade 5 ALT elevation, and no patient met the Hy's law or drug-induced liver injury (DILI) criteria. The majority of events resolved (374/185 events, 97.14%), while 2 more (0.52%) were reported as recovering/resolving which means that 2.34% did not resolve.

Infusion reactions: Less than 40% of patients (59/151 (39.1%) in the All-Treated Population and 53/141 (37.6%) in the Proposed Label Population) experienced at least 1 potential infusion-associated event. These events appeared in the first 48 hours following BMN 270 administration. 55 (60.4%) infusion-associated events reports in the Proposed Label Population were assessed as related to treatment with Roctavian. Following serious events (Grade 2 maculopapular rash/presyncope and Grade 3 hypersensitivity) observed in clinical trial 270-301, the protocol was amended to change the starting infusion rate to 1 mL/min instead of 4 mL/min, and adjustment (gradually increased up to 4 mL/min / interruption of infusion rate of infusion) to ensure that patients tolerate the infusion.

Thromboembolic events: Several subjects have shown FVIII activity above the ULN many of which until data cut-off. One of those patients required anti-platelet treatment, which was also still ongoing at data cut-off. No event or symptom suggestive of thromboembolism was observed as of the data

cut-off. However, a potential increased risk of thrombosis associated with increased levels of FVIII due to overexpression of the transgene resulting in supra-physiological FVIII activity levels (> 150 %) could not be excluded. The risk of thromboembolism is consequently considered as an important potential risk.

Development of anti-FVIII inhibitors (neutralizing antibodies): No patient in any BMN 270 study developed neutralizing antibody to FVIII as of the data cut-off.

Serious adverse events and deaths: A total of 59 SAEs were reported in 32 patients in the All Treated Population, and less than 20% of the patients treated at the therapeutic dose experienced at least one SAE. Only 8 SAEs in 7 patients were assessed as related to treatment with Roctavian with 2 serious Grade 3 ALT increased and 6 serious infusion-associated events (hypersensitivity in 2 patients, maculopapular rash and presyncope, pyrexia, anaphylactic reaction) in 5 patients.

3.5. Uncertainties and limitations about unfavourable effects

The safety profile of Roctavian is difficult to evaluate because of multiple factors: lack of a control arm, limited data on long term follow-up, impact of additional different approaches of corticosteroid therapy and non-clinical findings related to integration of AAV.

Hepatotoxicity

The management of ALT elevation by oral corticosteroids evolved in clinical trials, making difficult the assessment of the hepatoxicity events and the consequence of corticosteroid use. However, it is noted that ALT elevation was considered resolved more than 30 days after its onset in several patients and up to 488 days in others despite the use of corticosteroids. Moreover, some patients had additional ALT elevation events despite the ongoing corticosteroid treatment, and some patients treated with 6E13 vg/mL BMN 270 were treated for at least 6 months with corticosteroids. A potential chronicity of liver damage with a persisting need of immunosuppressive therapy could not be excluded.

Due to limited numbers of patients who received early/prophylactic versus therapeutic corticosteroids, data submitted so far are not sufficient to determine with certainty the best corticosteroid regimen (in terms of initiation, dose and duration of corticosteroid treatment) to facilitate transduction, achieve optimal long-term FVIII expression and reduce the early inflammatory response to Roctavian with the least corticosteroid-related side effects. Nevertheless, the applicant proposes a shorter and optimised reactive corticosteroid regimen associated with enhanced proactive hepatic monitoring in the SmPC, which will be put into perspective with the data provided via ongoing study 270-303 (supported by studies 270-203, 270-205) imposed as specific obligation to the CMA where patients will receive a prophylactic corticosteroid regimen.

Tumorigenic effects

The integration site analysis study performed on liver biopsies of cynomolgous monkeys showed low level of BMN 270 vector integration. Vector integration into human liver was detected in all five liver biopsies available from 5 different subjects. Integration was observed in other body cells than liver cells through causality assessment (concluded as not related) of a cancer in parotid gland in one patient treated with Roctavian in a clinical study. While AAV are not expected to integrate their genome in host cells at high frequency, all integration events could contribute to tumoral transformation.

Women of childbearing potential

In absence of available data in female at non-clinical (and clinical levels), the section 4.6 of the SmPC indicates that "ROCTAVIAN is not recommended for women of childbearing potential" excluding de

facto this subgroup. The section 4.6 of the SmPC provides the reasons for the recommendation not to use in WOCBP (i.e. could be harmful for the new-born child (theoretical risk of viral vector integration in foetal cells through vertical transmission) and that no data are available to recommend a specific duration of contraceptive measures in treated women of childbearing potential. The final report from a reproductive and developmental toxicity study conducted in wild type CD-1 female mice, to inform on the impact of BMN 270 on embryo-fetal developmental toxicity in females of childbearing potential and to establish an adequate waiting period after Roctavian infusion following which female patients can safely become pregnant, is expected by the end of December 2023 (as reflected in the RMP).

3.6. Effects Table

Effects Table for Roctavian in the treatment treatment of severe haemophilia A (congenital factor VIII deficiency) in adult patients without a history of factor VIII inhibitors and without detectable antibodies to adeno associated virus serotype 5 (AAV5). (data cut-off: 15.11.2021)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
FVIII activity level	Mean (SD) FVIII activity level at Week 104 (CSA)	IU/dL	22.7 (32.8)	N/A	ITT population (N=134) Large range of values (0.0-187.1) Limited duration of follow-up	Study 270-301
	Short Description	**Unit**	**Baseline**	**Post-FVIII prophylaxis period**	**Uncertainties/ Strength of evidence**	
Utilisation of Exogenous FVIII	Mean (SD) Infusion rate	infusions/ year	135.9 (52.0)	2.6 (8.5)	Rollover population (N=112) Large range of values, limited duration of follow-up	
ABR	Overall mean (SD) ABR for bleeds treated with exogenouos FVIII replacement	bleeds/ year	4.8 (6.5)	0.8 (2.4)		
Unfavourable Effects						
AE SAE			All patients N=59 in 32 patients	N/A	All Treated population (N=151) Limited duration of follow-up	Studies 270-301 270-201 270-203 270-302
ALT elevation		%	86,5%	N/A	Proposed Label population (N=141) Limited duration of follow-up Impact of corticosteroid use difficult to assess	Studies 270-301 and cohort 6E13 of 270-201

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
FVIII inhibitor			None	N/A	Limited duration of follow-up	
Non-clinical and clinical integration of AAV			Reported in animals and humans	N/A	Limited duration of follow-up	

Abbreviations: ABR: annualised of bleeding rate, DCO: data cut-off, ITT: intended to treat, AE: adverse event, SAE: serious adverse event, ALT: alanine aminotransferase

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

In the pivotal phase III study, the great majority of subjects (75,4%) develop endogenous FVIII production after a single infusion with a dose of 6E13 vg/kg and had a median FVIII activity level ⩾ 5 IU/dL at week 104 corresponding to FVIII level of at least mild haemophilia. This was globally translated in clinical relevant effects at week 104 on ABR (85.5% reduction) and FVIII utilisation (97.5% reduction) that could be considered as an important improvement over the standard therapy. Even in the low-responders, a reduction of ABR was observed in 62.5% of patients and the utilisation of exogenous FVIII was reduced by ⩾90% in 75% of the patients. These results are in favour of a potential clinically relevant benefit of Roctavian in HA patients that would reach FVIII activity levels <5 IU/dL. Based on the current safety database, the safety profile appears acceptable as the majority of patients experienced only Grade 1 (mild) or Grade 2 (moderate) TEAEs.

However significant uncertainties remain:

- **There is a lack of comprehensive data to support an adequate corticosteroid regimen**

The applicant has obviously had particular difficulties for determining the CS regimen to be recommended in the pivotal study and consequently in the SmPC. Indeed, the CS regimen has been changed at repeated times with corresponding amendments. Since the enrolment in the pivotal study has been completed, no subject is expected to have been treated with the recommended regimen during the 2 years follow up. More globally given the numerous changes on the CS regimen implemented during the study, subjects have received a mix of different CS regimen making quite difficult to enable substantiating the CS to be recommended. Therefore, as a significant caveat, no comprehensive data are currently available to determine the adequate CS regimen that could mitigate ALT increase and preserve FVIII activity while minimising AEs related to corticotherapy. The applicant is obviously further investigating this issue and while recommending reactive regimen, is still willing to collect additional data with a prophylactic regimen through a dedicated study 270-303 and the ongoing studies 270-203 (patients with anti-AAV5) and 270-205 (patients with FVIII inhibitors). Results of these studies will need to be put into perspective with the data provided via the registry, where patients will receive an optimised reactive CS regimen and with the data provided from the pivotal study 270-301.

- **There is a lack of comprehensive data to support a long term duration beyond the two years follow up of the pivotal 270-301 study**

Indeed, 5 years follow up is only available for the 7 patients (at the intended 6E13 vg/kg dose)

enrolled in the supportive study 270-201. Moreover, as requested, the applicant has developed a model to predict durability beyond 2 years. The model will have to be enriched with the longer term data from study 270-301 to achieve reliability in the prediction.

Therefore, comprehensive data to determine the adequate corticosteroid regimen and to further support a long-term duration beyond 5 years will have to be submitted as specific obligations in the context of the conditional marketing authorisation.

Overall, considering the burden of the corticosteroids associated to the administration of Roctavian with potentially a prolonged duration of CS course implying related CS adverse reactions and potential drug interactions with existing medications, the uncertainties of the long-term benefit of Roctavian beyond 2 years, the frequent hepatic function and FVIII activity monitoring associated to the administration of Roctavian, the quality of life that albeit expected to be improved by the gene therapy could nevertheless be worsen in relation to the burden of CS regimen and the potential risk of tumorigenesis, it is of major importance that a shared decision-making between physicians and patients applies (as underlined by the Ad Hoc Experts Group consulted) with regard to the initiation of Roctavian. This issue is addressed in section 4.4 of the SmPC and corresponding section of the PL as well as through and educational material for physicians and patients.

In addition, the lack of identified factors of variability of interpatient FVIII activity level prevents the identification of patients that could have a benefit of this treatment. This may lead to expose patients to a corticosteroid burden with no treatment benefit. Therefore investigations on predictive factors of no or low response are to be pursued as part of the Specific Obligation to the CMA with data derived from ongoing studies 270-203, 270-205 and 270-303.

Finally, the non-clinical and clinical findings on AAV integration is a source of particular concern insofar that it could contribute to a potential risk of tumorigenicity. The applicant has covered this potential identified risk as part of the safety concern of the RMP and with a total duration of follow up of 15 years as proposed in a PASS study.

Physicians and patients will be informed through warnings in the SmPC and the Patient leaflet and in guides as part of risk minimisation measures on the risk of vector integration in liver cells but also in other body cells (as observed through the information gained from causality investigation on a parotid gland tumour in a patient from the clinical study, determined as unrelated to Roctavian) and the risk of malignancy.

Uncertainties remain on the mechanisms involved in variability of FVIII expression and gradual FVIII levels decline despite the 5 liver biopsies provided by the applicant. It is of importance that results of liver biopsy substudies are submitted once available as part of the submissions for study 270-301 or other studies.

3.7.2. Balance of benefits and risks

The benefit/risk is considered positive.

3.7.3. Additional considerations on the benefit-risk balance

Comprehensive efficacy and safety data are lacking in the current MAA.

- Efficacy: duration of efficacy

The FVIII activity levels decrease over time question the durability of the effects that remains unpredictable. It remains unknown if the FVIII activity decrease might be sufficient to support hemostatic efficacy for multiple years or decline below the threshold of 5 IU/dL or return to baseline

value. In addition the limited data from the liver biopsies (N=5) up to 4 years after BMN 270 treatment from Study 270-201 does not supported a correlation between the durability of presence of stable, circular episomes of the FVIII transgene and the FVIII activity.

- Safety: length of follow up

The provided database for the clinical safety evaluation of this MAA is limited by the lack of long-term safety follow-up for the pivotal study, given the nature of the treatment with long-lasting, potentially life-long effects after a single dose and considering (i) the limited data set, especially with respect to mid- and long-term data, (ii) the thromboembolic risk linked to the high variability of FVIII activity levels observed (iii) treatment induced, immune based hepatotoxicity and the associated long and recurring need for immuno-modulatory treatment (iv) the theoretical risk of insertional mutagenesis.

Since it was suggested that pre-existing immunity to AAV may be associated with reduced transgene expression, only patients without pre-existing immunity to the AAV5 capsid could be enrolled in the initial clinical development (study 270-201 and 270-301). A study (270-203) is ongoing in patients with severe HA and pre-existing AAV5 antibody (1 enrolled among the 10 planned subjects to date). Considering the lack of data, the approved indication do not cover patients with severe HA and pre-existing AAV5 antibodies. This will in practice require testing of pre-existing anti-AAV5 immunity with a validated test as mentioned in the SmPC.

In both studies as well, patients with inhibitors to FVIII were part of the exclusion criteria. As a consequence, the approved indication do not cover those patients.

Conditional marketing authorisation

As comprehensive data on the product are not available, a conditional marketing authorisation was proposed by the CAT during the assessment, after having consulted the applicant.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it aims at the treatment of a seriously debilitating disease. In addition, the product is designated as an orphan medicinal product.

Furthermore, the CAT considers that the product fulfils the requirements for a conditional marketing authorisation:

- The benefit-risk balance is positive, as discussed.
- It is likely that the applicant will be able to provide comprehensive data coming from ongoing studies 270-301, 270-303, 270-203 and 270-205. The applicant provided 2-year efficacy data from the pivotal study 270-301 showing a FVIII activity decrease over the time leading to an uncertainty on the long-term efficacy. In addition, the corticosteroids to be administered to patients further to Roctavian administration were based on a reactive regimen in the pivotal study (i.e. in response to ALT increase) and only a limited number of patients from supportive study received a prophylactic corticosteroids use (i.e. before ALT increase), questioning on the most adequate CS regimen to facilitate transduction, achieve optimal long-term FVIII expression and reduce the early inflammatory response to Roctavian with the least AE/SAE. The two specific obligations below are associated to the conditional MA to address the lack of comprehensiveness:

    In order to confirm the efficacy and safety of Roctavian in adults with severe haemophilia A (congenital factor VIII deficiency) without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5), the MAH should submit the final results including 5 years follow-up of the phase 3, single arm study 270-301.

    In order to confirm the efficacy and safety of Roctavian, adequate corticosteroid regimen and to identify predictive factors for no or low response in adults with severe haemophilia A

(congenital factor VIII deficiency), the MAH should submit the final results of the phase 3 single arm study 270-303 in patients receiving a prophylactic corticosteroid regimen. Interim data from open- label studies 270-203 and 270-205 should also be provided.

- Unmet medical needs will be addressed, as Roctavian requires a single intravenous administration that could free severe HA patients from therapeutic burden for at least 2 years (few patients from the phase 2 study 201 have achieved 5 years) while the available treatment options require a variable number of injections, i.e. prophylactic infusion of exogenous FVIII two to three times per week, or episodically at the time of a bleeding event, and repeat administration every 1-4 weeks for emicizumab (in patients with or without FVIII inhibitors).
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. As benefit-risk balance on basis of the current data is regarded positive, an additional therapy option for severe haemophilia patients is considered beneficial.

The CHMP endorses the CAT conclusion on conditional marketing authorisation as described above.

3.8. Conclusions

The overall benefit/risk balance of Roctavian is positive, subject to the conditions stated in section 'Recommendations'.

The CHMP endorse the CAT conclusion on the benefit-risk balance as described above.

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the benefit-risk balance of Roctavian is favourable in the following indication(s):

Roctavian is indicated for the treatment of severe haemophilia A (congenital factor VIII deficiency) in adult patients without a history of factor VIII inhibitors and without detectable antibodies to adeno associated virus serotype 5 (AAV5).

The CAT therefore recommends the granting of the conditional marketing authorisation subject to the following conditions. Based on this draft opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Roctavian in the treatment of severe haemophilia A (congenital factor VIII deficiency) in adult patients without a history of factor VIII inhibitors and without detectable antibodies to adeno associated virus serotype 5 (AAV5) is favourable and therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of PSURs for this medicinal product are set out in Article 9 of Regulation (EC) No 507/2006 and, accordingly, the marketing authorisation holder (MAH) shall submit PSURs every 6 months.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Prior to launch of Roctavian in each Member State, the marketing authorisation holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The MAH shall ensure that in each Member State where Roctavian is marketed, all healthcare professionals and patients, carers and observers who are expected to prescribe, use or oversee the administration of Roctavian have access to/are provided with the following educational package. These documents will be translated in the local language to ensure understanding of proposed mitigation measures by physicians and patients:

- Physician Educational Material
- Patient Information Pack

The physician educational material should contain:

- The Summary of Product Characteristics
- Guide for healthcare professionals
- The patient guide
- The patient card

The guide for healthcare professionals:

- Patient selection: patients should be selected for treatment with Roctavian based on the absence of antibodies to AAV5 using an appropriate validated assay and status of liver health based on laboratory and imaging data.
- To inform of the important identified risk of hepatotoxicity and the important potential risks of horizontal and germline transmission, development of factor VIII inhibitors, malignancy in relation to vector genome integration, and thromboembolism, and details on how these risks can be minimised.
- Before a treatment decision is made, the healthcare professional should discuss the risks, benefits, and uncertainties of Roctavian with the patient when presenting Roctavian as a treatment option, including:
    - That no predictive factors for no or low responders have been identified. Patients who do not respond are still exposed to long-term risks.
    - That the long-term treatment effect cannot be predicted.
    - That there would be no plans to re-administer the medicinal product for patients who do not respond or have lost the response.
    - Reminding patients about the importance to enrol in a registry for follow up of long-term effects.
    - That Roctavian use will require in most cases co-administration of corticosteroids to manage the liver damage that this medicinal product might induce. This requires adequate

monitoring of patients and careful consideration of other co-medications, to minimise the risk of hepatoxicity and a potential reduced therapeutic effect of Roctavian.

The patient information pack should contain:
- The patient information leaflet
- The patient guide
- The patient card

The patient guide:
- Importance to fully understand the benefits and risks of Roctavian treatment, what is known and not yet known about the long-term effects, related to both safety and efficacy.
- Therefore, before a decision is made about starting on the therapy the doctor will discuss with the patient the following:
    - That not all patients may benefit from treatment with Roctavian and the reasons for this have not been established. Patients not responding to treatment will still be exposed to long-term risks.
    - That Roctavian will, in most cases, require co-treatment with corticosteroids to overcome the liver damage that this medicine may produce, and that the doctor will ensure that patients are available for regular blood tests to check response to Roctavian and assess liver health. Patients should inform the healthcare professional about current use of corticosteroids or other immunosuppressants. If the patient cannot take corticosteroids, the doctor may recommend alternative medicines to manage problems with the liver.
    - That Roctavian has a viral vector component, and it may be associated with an increased risk of malignant tumour.
    - Details how the important identified risk of hepatotoxicity and the important potential risks of horizontal and germline transmission, development of factor VIII inhibitors, malignancy in relation to vector genome integration, and thromboembolism can be recognised and minimised by regular monitoring as recommended by doctors.
    - That the patient will get a patient card that should be shown to any doctor or a nurse whenever the patient has a medical appointment.
    - The importance to participate in the patients' registry for long-term surveillance of 15 years.

The patient card:
- This card is to inform healthcare professionals that the patient has received Roctavian for haemophilia A.
- The patient should show the patient card to a doctor or a nurse whenever they have an appointment.
- The card should mention the specific mitigation measures to minimise the risks related to hepatotoxicity, horizontal and germline transmission, development of factor VIII inhibitors, malignancy in relation to vector genome integration, and thromboembolism.
- The card should warn healthcare professionals that the patient is likely undergoing treatment with corticosteroids for minimising the risk of hepatotoxicity with Roctavian.

The CHMP endorse the CAT conclusion on the additional risk minimisation measures.

- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date

In order to further characterise the long term efficacy and safety of Roctavian in adults with severe haemophilia A (congenital factor VIII deficiency) without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5), the MAH should conduct and submit the final results of study 270-401, a follow-up study of patients enrolled in the clinical studies.	31 July 2038
In order to further characterise the long term efficacy and safety of Roctavian in adults with severe haemophilia A (congenital factor VIII deficiency) without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5), the MAH should conduct and submit the final results of the study 270-801, a Retrospective Cohort Study of patients treated with valoctocogene roxaparvovec based on data from a registry, according to an agreed protocol.	30 June 2044
In order to further characterise the long-term efficacy and to further inform on the risk-benefit balance of Roctavian in adults with severe haemophilia A (congenital factor VIII deficiency) in a broader population, the MAH should conduct and submit the final results of the study 270-601.	30 September 2042

The CHMP endorses the CAT conclusion on the obligation to conduct post-authorisation measures as described above.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	**Due date**
In order to confirm the efficacy and safety of Roctavian in adults with severe haemophilia A (congenital factor VIII deficiency) without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5), the MAH should submit the final results including 5 years follow-up of the phase 3, single arm study 270-301.	30 June 2025
In order to confirm the efficacy and safety of Roctavian, adequate corticosteroid regimen and to identify predictive factors for no or low response in adults with severe haemophilia A (congenital factor VIII deficiency), the MAH should submit the final results of the phase 3 single arm study 270-303 in patients receiving a prophylactic corticosteroid regimen. Interim data from open- label studies 270-203 and 270-205 should also be provided.	30 September 2027

The CHMP endorses the CAT conclusion on the specific obligation to complete post-authorisation measures for the conditional marketing authorisation as described above.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the review of available data on the active substance, the CAT considers that valoctocogene roxaparvocec is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

The CHMP endorses the CAT conclusion on the new active substance status claim.